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Effect of an exercise and dietary intervention on serum biomarkers in overweight and obese adults with osteoarthritis of the knee[1]

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Summary

Objective—To determine the effects of exercise and weight loss interventions on serum levels of four biomarkers and to examine if changes in biomarker levels correlate with clinical outcome measures in obese and overweight adults with knee OA.

Methods—Serum was obtained at baseline, 6- and 18-months from 193 participants in ADAPT (Arthritis, Diet and Activity Promotion Trial). This was a single-blind 18-month trial with subjects randomized to four groups: healthy-lifestyle (HL), diet (D), exercise (E) and diet plus exercise (D +E). Serum levels of cartilage oligomeric protein (COMP), hyaluronan (HA), antigenic keratan sulfate (AgKS) and transforming growth factor-β1 (TGF-β1) were measured by ELISA.

Results—At baseline there were no significant differences in biomarker levels between intervention groups. When results for all the intervention groups were combined, the levels of HA were found to be negatively correlated with medial joint space width and positively correlated with Kellgren-Lawrence scores (K-L scores) while TGF-β1 levels negatively correlated with K-L scores. When biomarker levels measured at 6 and 18-months were adjusted for baseline values, age, gender, and body mass index (BMI), weak but significant differences between intervention groups were present for mean levels of COMP and TGF-β1. Furthermore, AgKS levels averaged over all groups tended to decrease over time. There were no significant associations of baseline biomarkers and the follow-up outcomes. Weak associations were noted between change in the biomarkers at 18-months and change in outcome measures that included change in weight with AgKS and COMP and change in WOMAC pain with AgKS.

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Conclusion—Overall, the exercise and dietary interventions did not show a consistent effect on levels of potential OA biomarkers. The four biomarkers showed differences in correlations with outcome measures suggesting they may measure different aspects of disease activity in OA. The strongest correlations were between serum HA and radiographic measures of OA at baseline.

Keywords

biomarkers; osteoarthritis; clinical trials; hyaluronan; COMP; TGF-β

Introduction

Osteoarthritis (OA) is a progressive, painful and often disabling disease characterized by the loss of articular cartilage, which is accompanied by bony hypertrophy within a diarthrodial joint. Unmet needs in clinical OA studies are the ability to detect early disease prior to radiographic changes, to determine or predict the rate of disease progression of the joint, and to rapidly ascertain whether or not a therapeutic intervention slows down or stops disease progression. These needs must be effectively addressed if the detection and management of OA is to be improved.

At present, plain radiographs are commonly used to classify OA subjects for the purposes of clinical studies and joint space narrowing is often used as a measure of disease progression. Although plain radiography is, at present, the "gold standard" for evaluation of OA progression, it is fraught with problems related to the accurate reproduction of measurements of joint space width, especially in subjects who have knee OA[1–3].

Recently, researchers have gained knowledge about the biology of OA and have identified molecular events that lead to the destruction and remodeling of joint tissues, including cartilage and bone[4,5]. Cytokines, such as interleukin-1 (IL-1) and IL-6 and other inflammatory mediators, are found within the cartilage and are thought to participate locally in cartilage destruction by inhibiting matrix synthesis and stimulating the release of degradative enzymes[6,7]. Specific degenerative and biosynthetic events, which have been identified and quantified by the use of antibody-based immunoassays, are used to study tissue-specific changes in OA as reflected by molecular biomarkers[5,8,9].

Among the most promising of these biomarkers are type II collagen degradation products, antigenic keratan sulfate (AgKS) epitopes, cartilage oligomeric matrix protein (COMP), GP-39/YK-40, type 1 collagen cross-links, several matrix metalloproteinases, and hyaluronan (HA)[5,9,10]. The biomarkers can be detected principally in the joint tissues where the events occur, but also in body fluids, such as peripheral blood, urine and synovial fluid, into which the biomarkers are released. Three of the most commonly used serum biomarkers associated and/or correlated with OA and joint progression are: AgKS[11,12], HA[11,13–15], and COMP[16–19]. Although not as well studied, TGF-β1 levels measured in the serum of OA subjects were found, out of 14 serum and urine biomarkers tested, to be best associated with a change in clinical assessments over a 1-year period[20].

While most biomarker studies attempt to distinguish patients who have OA from the non-arthritic population, a few studies have used biomarkers to predict progression[11,13–18,21,22]. Most of these studies are limited by their cross-sectional nature, and those that are longitudinal are observational. Hence, there is little data available on the effects of a therapeutic intervention on changes in biomarker levels.

The objective of the present study was to determine the correlation of selected biomarkers with clinical outcome measures in an intervention study using serum samples collected from the

ADAPT (Arthritis, Diet and Activity Promotion Trial) participants. ADAPT was an 18-month single blind randomized clinical trial designed to determine if exercise and dietary weight loss, alone or in combination, were more effective than usual care in improving pain and function in older overweight and obese adults with knee OA[23]. Both groups receiving the dietary intervention lost on average about 5% body weight and the primary outcome, self-reported physical function ascertained by WOMAC, showed significant improvements of 24% in the diet plus exercise group and 18% in the diet only group. The present study reports data on the effects of the interventions on serum biomarkers, specifically: AgKS, HA, COMP and TGF-β1, and their correlations with outcome measures.

Methods

PARTICIPANTS

Details of the ADAPT study were previously published[23,24]. Briefly, 316 patients, aged 60 years and older with a body mass index (BMI) of 28 and above, who had clinical evidence of knee OA and who met the study criteria, were assigned to one of the four therapeutic intervention cohorts: healthy lifestyle (HL, control group), diet (D), exercise (E) and diet plus exercise (D+E). The HL intervention consisted of a regular group meeting to provide attention, social interactions and health education. The diet intervention was a behavior modification type intervention designed to produce a group average of 5% weight loss. The exercise intervention consisted of a combination of aerobic (walking) and resistance-training for a total of one hour three times a week. Participants in the D+E group received a combination of the D and E interventions.

For all interventions, self-reported physical function (WOMAC function, pain and stiffness), measures of mobility (stair climb time and six-minute walk distance), weight loss and knee radiographs (medial joint space width and K-L score) were the outcome measures used as previously described in detail[23]. Outcome measures were determined at baseline, 6-months and 18-months except for the radiographs which were obtained only at baseline and 18-months.

BIOMARKER MEASUREMENTS

After the participants had fasted overnight, blood was collected by venipuncture in the morning (between 07:00 AM and 09:00 AM) at baseline and after 6- and 18-months of intervention. Serum was frozen at −80°C until analyzed. Of the 316 subjects in ADAPT, sufficient serum for biomarker measures was available from all 3 time points in 193 subjects. In the present study, the clinical and radiological outcomes were analyzed for these 193 subjects. Hyaluronan was measured using a well-characterized sandwich enzyme linked immunosorbent assay (ELISA) technique[25]. Briefly, the procedure utilizes an anti-KS monoclonal antibody (1/20/5-D-4; MP Biomedicals, Irvine, CA) to differentiate between the coated aggregating rat chondrosarcoma proteoglycan, which captures HA, and the AgKS-bearing aggregating proteoglycan added subsequently. Antigenic keratan sulfate was quantified by a previously described ELISA technique[26,27] that includes an inhibition step and also utilizes monoclonal antibody 5-D-4.

Cartilage oligomeric matrix protein was measured using the AnaMar COMP ELISA (AnaMar Medical AB Bangardsgatan, Uppsala, Sweden). The COMP ELISA is a solid-phase, two-site enzyme immunoassay, based on a direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the COMP molecule[28]. The TGF-β1 level was measured using a highly sensitive ELISA (Quantikine Human TGF-β1 kit from R&D Systems Inc., Minneapolis, MN). This assay also employs the quantitative sandwich enzyme immunoassay technique and uses an anti-TGF-β1 monoclonal antibody and a polyclonal antibody against TGF-β 1 conjugated to horseradish peroxidase[29].

All samples were measured in duplicate, and the average of the two values was used for data analyses. Duplicate samples that did not provide a co-efficient of variation <15% were reanalyzed. The intra-assay and inter-assay variation of the tests are as follow: HA (intra: <4% and inter: <6%); KS (intra: <3% and inter: <4%); COMP (intra: 1.7–3.0% and inter: 1.8–4.2%); and TGF-B-1 (intra: 3.7% and inter: 9.8–12.8%).

STATISTICAL ANALYSIS

Statistical analyses were performed using SAS software version 8 (SAS Institute, Cary, NC). A significance level of 0.05 was adopted for all comparisons. Descriptive statistics were calculated for each intervention group (HL, D, E and D+E) at baseline. Values were reported as means ± standard error (SE) unless otherwise indicated. Logarithmic transformations of the biomarker results to satisfy the model assumptions (normally distributed errors and linear relations) were tested, however, the results did not change when compared to non-log transformed values. Hence, non-transformed data were reported. Analysis of variance and chi-square tests were used to determine differences among baseline characteristics between intervention groups.

The effects of diet and or exercise programs on disability, physical function, pain, and measures of mobility measured at 6- and 18-months post-randomization were determined by repeated measures analysis of covariance. All follow-up information was analyzed using SAS PROC MIXED (SAS Institute, Cary, NC). Analyses of group differences were adjusted for the pre-randomization levels of baseline values of the outcome being analyzed and by age, gender and BMI. A random effect of subjects that accounted for the within-subject correlation at the repeated measurements was included. Estimates of intervention effects were obtained at each follow-up observation. To test the consistency of intervention effects during the follow-up period, tests of time of follow-up by intervention effects were conducted. When time-by-intervention interactions were non-significant, average, intervention effects over the follow-up period were estimated and tested for significance.

Pearson's correlation coefficients were used to examine the relationship between biomarkers and BMI, WOMAC function, WOMAC pain, WOMAC stiffness, six-minute walk distance, stair climb-time, joint space width and the K-L score at baseline. Repeated measures analysis of covariance was also used to investigate the relationship between outcome measures and baseline biomarkers. The same covariates as above were included in the models, except that BMI was not added to the model for weight. The slope for each biomarker is reported.

Results

EFFECTS OF TIME AND GROUP ASSIGNMENT ON BIOMARKER LEVELS

Analyses were conducted on data from 193 ADAPT subjects with a baseline blood sample and any follow-up (6 and 18 month) samples. There were no significant differences in any baseline characteristics of these subjects (Table 1). When examining mean baseline outcome values for all subjects tested, participants in the diet group tended to have lower WOMAC function (p=0.02) pain (p=0.03) and stiffness (p=0.01) (Table 2). All 4 groups showed similar levels of biomarkers at baseline (Figure 1).

Biomarker levels remained relatively stable during the 18-months of the study (Figure 1 and Table 3), except for AgKS levels which, averaged over all groups, decreased significantly over time (p=0.02). Statistically significant differences between groups were present for mean levels of COMP (p=0.04) and TGF-β1 (p=0.02) (Table 3). Pair-wise comparisons revealed that participants in the exercise group had significantly higher levels of COMP than those in the diet and exercise group (p=0.01) and in the control group (p=.03). TGF-β1 levels in the diet

group were significantly lower than in the control group (p=0.003) and in the diet and exercise group (p=.04). These group differences did not vary significantly over time.

OUTCOME MEASURES AND LEVELS OF BIOMARKERS

There were no statistically significant correlations between baseline outcome measures and baseline levels of biomarkers (Table 4), except for KL-scores which were positively associated with HA (r=0.35, p=0.0001) and negatively correlated with TGF-β1 (r=−0.19, p=0.01). In addition, medial joint space width (JSW) was also associated with HA (r=−0.23, p=0.004). Slopes for the associations between follow-up outcomes and baseline levels of biomarkers tended to be small and statistically non-significant (Table 5). Only weight was significantly associated with AgKS (slope =0.02, p= 0.01).

There were a few weak but significant associations between the change in biomarker levels and change in outcome measures at 18-months (Table 6). These were for change in weight with change in AgKS (p=0.03) and COMP (p=0.02) and for change in WOMAC pain and change in AgKS (p=0.04).

Discussion

There is very little data in the literature about the effects of therapeutic interventions on biomarker levels in an OA population and how outcome measures commonly used in clinical trials might correlate with these levels. In this study of participants in an exercise and weight loss intervention, we found that the serum levels of HA, COMP, and TGF-β1 remained relatively stable during the 18-month intervention period while there was an overall slight decline in AgKS. Any differences observed between intervention groups with quite minimal.

The most consistent and strongest correlations noted in the present study between biomarker levels and clinical outcome measures were noted with HA and radiographic measures of OA. Serum HA levels at baseline were negatively correlated with medial joint space width and were positively correlated with the K-L score. These findings are consistent with other studies that have shown similar correlations between HA levels and radiographic OA[13–15,30]. Serum levels of HA in people with knee OA are thought to reflect the level of synovial inflammation because HA is produced by synovial cells and inflammation in the synovium may allow greater amounts to enter the systemic circulation[5].

Our study also showed a significant but weak negative correlation between baseline TGF-β1 levels and K-L score. TGF-β has anabolic effects on cartilage that might account for a negative association with systemic levels and K-L score, but it has also been implicated in osteophyte formation in OA[31,32]. Separate osteophyte scores would have been of interest to correlate with levels of TGF-β but were not available. Our results failed to confirm previous work suggesting that serum TGF-β might serve as a marker for clinical outcomes in OA[20].

The effect of weight loss on OA biomarkers has not been previously studied and most reports on the effects of exercise have been on biomarkers in healthy athletes or endurance runners. Sweet et al. found no differences in the serum levels of AgKS in marathon runners before and after the completion of a marathon run[33] and showed that serum AgKS levels decreased during periods of immobilization, but rose after return to ambulation[34]. Serum levels of COMP have been shown to increase just after exercise and return to baseline levels after 30 minutes and with no increase in baseline levels noted after 6 weeks of an exercise program[35]. In two recent studies using serum samples from the ADAPT subjects, we have shown that the dietary weight loss but not the exercise intervention significantly reduced levels of the inflammation markers, C-reactive protein, interleukin-6, and soluble tumor necrosis factor α receptor 1[36], and reduced

the levels of the adipokine leptin[37]. In the present study, only weak correlations were noted with change in AgKS and COMP and change in weight over18-months.

Although serum COMP has been widely studied as a potential OA biomarker[5], we did not find that COMP levels at baseline, 6- or 18-months correlated with either subjective or objective outcome measures other than the change in weight. In the Johnston County Osteoarthritis Project, levels of COMP varied by ethnicity and sex and were associated with age, BMI and radiographic OA[38]. Our study did not find any significant differences between the levels of COMP among our subject populations, which were predominantly Caucasian women. Of interest, a recent longitudinal observational study has shown an association of an increase in COMP with increased risk of cartilage loss on MRI in subjects with knee OA[39]. Our radiographic measure of joint space width on plain films would be unlikely to detect a similar association given the relatively small sample size of the current study and the inherent variability in joint space width measures.

There are several important limitations in the present study. When present, correlations with clinical outcomes, though statistically significant, were relatively weak. Because this was an exploratory study we examined all results with p values <0.05. Because multiple comparisons were examined, some of the apparent significant observations may have occurred by chance alone. Also, recent work has suggested that measurement of markers that reflect cleavage of type II collagen, such as CTX-II, may serve as better OA biomarkers than those measured here[22,40,41] although this is still controversial[42]. However, the most promising of these assays require measurements in urine and only serum samples were collected in the present study. Finally, as with most biomarker studies in OA, a major limitation is that a systemic measure is being correlated with local disease activity. About 55% of the subjects in the ADAPT study reported having arthritis in more than one joint[23] but the clinical and radiographic outcome measures focused on knee OA.

In conclusion, the present study showed that serum levels of four potential biomarkers were relatively stable during the 18-month intervention. Previous observations that worse radiographic knee OA correlates with higher HA serum levels were confirmed. Based on the recent suggested classification criteria of biomarkers (burden of disease, investigative, prognostic, efficacy of intervention, and diagnostic)[43]the only marker in the present study that could be classified would be HA as a marker for burden of disease. Although it was not possible to conclude from the biomarker measures that the diet and exercise interventions improved joint structures, the finding of little change in levels of the four markers over the course of the study is consistent with the premise that the interventions did not result in measurable harm to the joints. This was a potential concern with overweight and obese subjects in an exercise program that included weight-bearing exercises which result in periods of increased joint loading.

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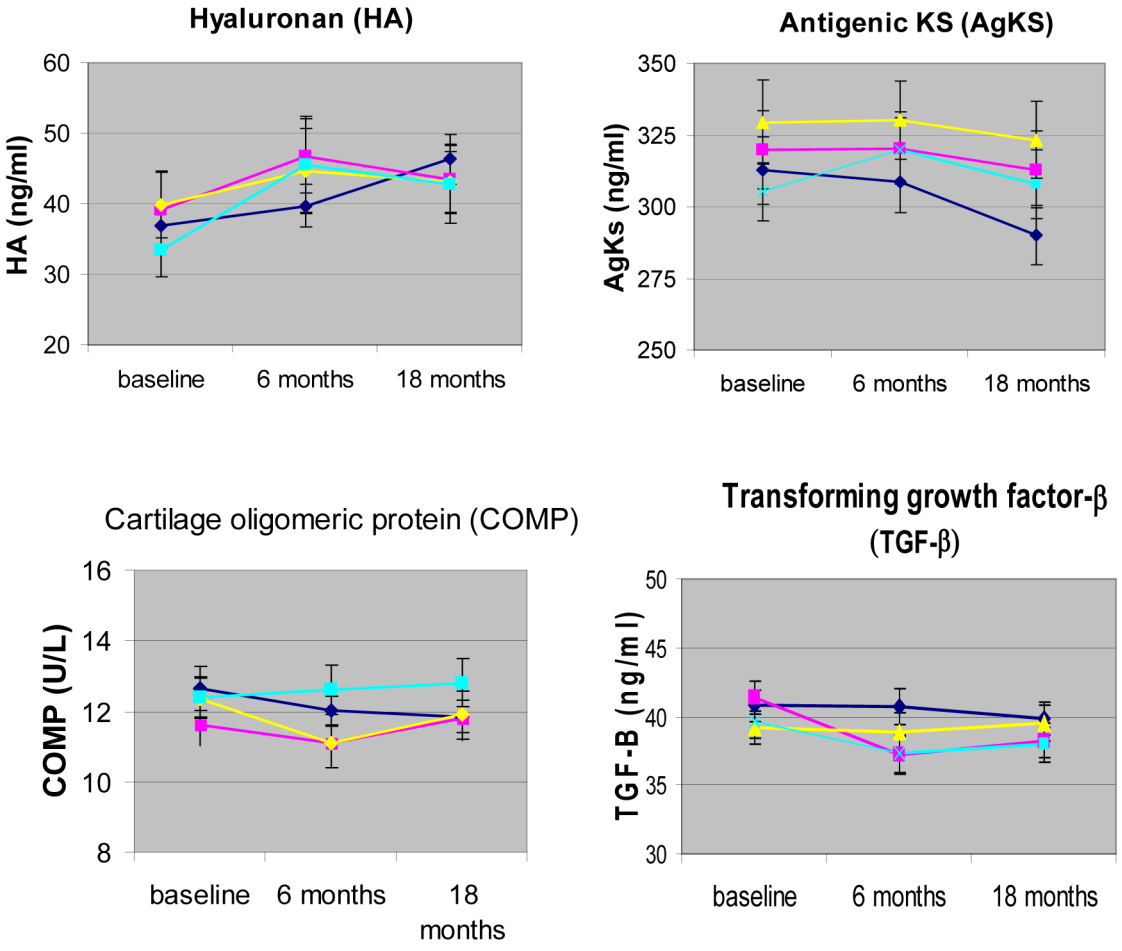

Fig. 1.

Biomarker levels by group assignment over the course of the study. The levels of the indicated biomarkers were measured by ELISA assays in serum samples obtained from ADAPT subjects at baseline, 6-months, and 18–months. ♦ = healthy life-style control; ■ = diet; ▲ = diet + exercise; ■ = exercise.

Table 1

Demographics of ADAPT Subjects Utilized for Biomarker Studies, by Intervention Arm

	Diet+Exercise N=46	Diet N=48	Exercise N=46	Control N=53	P-Value
	N (%)	N (%)	N (%)	N (%)	
Male	9 (19)	14 (30)	15 (32)	19 (38)	.24
White	35 (74)	39 (81)	38 (81)	45 (85)	.62
	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	
Age (years)	67.80 (0.95)	68.51 (0.83)	68.67 (0.86)	68.94 (0.83)	.82
Weight (lbs)	194.8 (4.6)	208.4 (5.0)	196.2 (4.3)	207.8 (5.3)	.08
BMI (kg/m^2)	33.10 (0.67)	33.64 (0.59)	33.65 (0.86)	34.26 (0.73)	.72

SE = Standard Error

Table 2

Outcome Measures at Baseline of ADAPT Subjects Utilized for Biomarker Studies, by Intervention Arm

	Diet+Exercise N=46	Diet N=48	Exercise N=46	Control N=53	P-Value
	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	
KL score	2.52 (0.14)	2.54 (0.11)	2.50 (0.13)	2.51 (0.13)	.99
WOMAC Function	23.38 (1.76)	17.76 (1.74)	25.33 (1.74)	22.42 (1.67)	.02
WOMAC Pain	6.33 (0.53)	5.11 (0.53)	7.39 (0.53)	6.42 (0.51)	.03
WOMAC Stiffness	3.53 (0.22)	2.78 (0.22)	3.87 (0.22)	3.36 (0.21)	.01
Distance walked (ft)	1472 (46.9)	1392 (38.7)	1535 (48.9)	1485 (44.6)	.17
Stair climb time (s)	9.14 (0.64)	9.12 (0.84)	9.90 (1.55)	9.85 (0.82)	.91
JSW_LA (mm)	4.89 (0.20)	4.70 (0.17)	4.63 (0.22)	4.72 (0.18)	.81
JSW_MED (mm)	3.34 0.24)	3.17 (0.21)	3.47 (0.22)	3.23 (0.24)	.80

KL= Kellgren-Lawrence; WOMAC = Western Ontario and McMaster Universities Osteoarthritis Index; ft = feet; s= seconds; JSW = joint space width; LA= lateral; MED=medial; mm=millimeters

Table 3

Biomarker levels measured at 6 months and 18 months, by intervention arms, adjusted for baseline values, age, gender and BMI, mean (SE)

	Diet+Exercise		Diet		Exercise		Control		P-value*	
Outcomes	6-Month N=46	18-Month N=46	6-Month N=48	18-Month N=48	6-Month N=45	18-Month N=45	6-Month N=52	18-Month N=53	Grp	Time
HA (ng/ml)	42.28 (3.79)	45.33 (3.63)	46.95 (3.64)	44.21 (3.44)	45.68 (3.67)	43.45 (3.54)	40.46 (3.58)	47.67 (3.35)	.99	.50
AgKS (ng/ml)	310.22 (7.62)	310.93 (7.32)	312.61 (7.29)	306.38 (6.90)	322.79 (7.35)	314.11 (7.09)	308.67 (7.17)	286.66 (6.71)	.07	.02
COMP (U/L)	10.80 (0.49)	11.81 (0.46)	11.58 (0.47)	12.21 (0.44)	12.77 (0.46)	12.84 (0.44)	11.75 (0.45)	11.72 (0.42)	.04	.10
TGFβ1 (ng/ml)	38.89 (1.14)	39.06 (1.07)	36.40 (1.08)	36.65 (1.01)	37.63 (1.08)	38.46 (1.04)	40.93 (1.04)	39.41 (0.98)	.02	.87

HA = hyaluronan, AgKS = antigenic keratan sulfate, COMP = cartilage oligomeric protein, TGF-β1 = transforming growth factor-B1.

*Interactions for Group and time not statistically significant.

Table 4

Correlation of baseline biomarker levels with baseline outcome measures, r (p value)

Outcomes	HA	AgKS	COMP	TGF-β1
WEIGHT	0.05 (.49)	0.04 (.60)	−0.08 (.29)	0.02 (.78)
WOMC function	0.12 (.11)	−0.03 (.67)	−0.07 (.36)	−0.14 (.07)
WOMC pain	0.09 (.21)	−0.12 (.10)	−0.04 (.62)	−0.09 (.22)
WOMC stiffness	0.11 (.12)	−0.07 (.36)	−0.08 (.31)	−0.08 (.30)
Distance walked	−0.02 (.78)	0.09 (.27)	0.15 (.06)	−0.03 (.71)
Stair climb time	0.05 (.54)	−0.13 (.07)	−0.11 (.14)	−0.09 (.22)
JSW lateral	−0.08 (.32)	0.11 (.14)	−0.13 (.10)	−0.13 (.10)
JSW medial	−0.23 (.004)	0.02 (.83)	−0.06 (.45)	0.07 (.38)
K-L score	0.35 (.0001)	0.06 (.46)	0.01 (.89)	−0.19 (.01)

HA = hyaluronan, AgKS = antigenic keratan sulfate, COMP = cartilage oligomeric protein, TGF-β1 = transforming growth factor- β1, JSW = joint space width. Values shown are correlation coefficients followed by p values in parentheses.

Table 5

Association between baseline biomarker levels and outcome measures, adjusting for age, gender, BMI (except for weight), baseline value, group and visit.

Outcomes	HA	AgKS	COMP	TGF-β1
WEIGHT	−.003 (.023) .90	.02 (.01) .01	.12 (.16) .46	.02 (.08) .78
WOMC function	−.0004 (.001) .77	−.001 (.0004) .16	−.01 (.01) .24	.0005 (.005) .92
WOMC pain	−.001 (.001) .38	−.003 (.001) .50	−.01 (.01) .49	−.003 (.005) .46
WOMC stiffness	.00002 (.002) .99	−.001 (.001) .03	−.01 (.01) .22	−.004 (.01) .45
Distance walked	.58 (.63) .36	.13 (.20) .53	−6.5 (4.24) .13	−.71 (2.00) .72
Stair climb time	−.01 (.01) .19	.01 (.004) .08	−.08 (.08) .30	−.03 (.04) .49
JSW lateral	−.001 (.002) .67	.0002 (.001) .74	.02 (.01) .17	−.01 (.01) .52
JSW medial	−.004 (.002) .10	.001 (.001) .46	.01 (.01) .55	.01 (.01) .26
K-L score	.001 (.001) .34	−.0003 (.0003) .35	.01 (.01) .40	−.002 (.004) .54

HA = hyaluronan, AgKS = antigenic keratan sulfate, COMP = cartilage oligomeric protein, TGF-β1 = transforming growth factor- β1, JSW = joint space width. Values shown are Beta coefficients (SE) followed by p values.

Table 6

Association between change in biomarker levels at 18-months and change in outcome measures 18-months. Beta coefficient (SE) and p-value

	HA-Chg	AgKs-Chg	COMP-Chg	TGF-Beta1-Chg
Outcomes				
1.WEIGHT-Chg	0.05 (.04) P=.20	0.04 (.02) P=.03	0.68 (.29) P=.02	−0.02 (.15) P=.89
2.WOMCFUNC-Chg	0.004 (.003) P=.12	−0.002 (.001) P=.15	−0.03 (.02) P=.13	0.01 (.01) P=.92
3.WOMCPAIN-Chg	0.001 (.003) P=.64	−0.003 (.001) P=.04	−0.03 (.02) P=.15	0.02 (.01) P=.79
4.WOMCSTIF-Chg	0.001 (.003) P=.69	−0.0003 (.001) P=.85	−0.03 (.02) P=.25	0.003 (.01) =.79
5.DISTANCE-Chg	0.55 (1.24) P=.66	−0.03 (.60) P=.96	−0.076 (9.9) =.94	−5.5 (5.1) P=.28
6.STCLTIME-Chg	−0.01 (.03) P=.56	0.006 (.01) P=.60	−0.23 (.20) P=.26	−0.02 (.11) =.85
7.JSW_LA-Chg	−0.0001 (.002) p=.98	0.0001 (.001) P=.91	0.04 (.02) P=.05	.003 (.01) =.81
8.JSW_MED-Chg	−0.004 (.002) P=.08	0.001 (.001) P=.65	−0.02 (.02) P=.34	−0.004 (.01) P=.68
9.SCORE-Chg	0.001 (.001) P=.70	−0.0003 (.001) =.60	−0.001 (.01) P=.92	−0.01 (.01) P=.12

HA = hyaluronan, AgKS = antigenic keratan sulfate, COMP = cartilage oligomeric protein, TGF- β1 = transforming growth factor- β1, JSW = joint space width. Values shown are Beta coefficients (SE) followed by p values.