Associations of urinary biomarker COLL2-1NO2 with incident clinical and radiographic knee OA in overweight and obese women

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Abstract

Objective: To investigate the association between urinary biomarker Coll2-1NO_2 (uColl2-1NO_2) and incident knee OA after 2.5 years follow-up in middle-aged overweight and obese women at high risk for knee osteoarthritis (OA).

Design: Data were used from PROOF, a randomized controlled trial with 2.5 years follow-up evaluating the preventive effects of a diet and exercise program and oral glucosamine sulphate (double blind and placebo controlled), on development of incident knee OA in women with body mass index ≥ 27 kg/m^2 without signs of knee OA at baseline. Baseline and 2.5 years uColl2-1NO_2 concentrations were assessed with ELISA. Primary outcome measure was incidence of knee OA in one or both knees, defined as incidence of either Kellgren & Lawrence grade ≥ 2, joint space narrowing of ≥ 1.0 mm or knee OA according to the combined clinical and radiographic ACR-criteria. We used binary logistic regression for the association analyses.

Results: 254 women were available for analyses. At 2.5 years follow-up, incident knee OA was present in 72 of 254 women (28.3%). An inversed association was found between baseline uColl2-1NO_2 and incident knee OA at 2.5 years (OR 0.74, 95% CI 0.55 – 0.99*)*. The concentration at 2.5 years and the change in concentration over 2.5 years did not show significant associations with the outcome.

Conclusions: In overweight and obese middle-aged women, not higher but lower baseline uColl2-1NO_2 concentration was significantly associated with an increased risk for incident knee OA. This interesting but counterintuitive outcome makes further validation of this biomarker warranted.

Keywords knee, osteoarthritis, biomarker, Coll2-1NO_2

Introduction

Up to now there is no curative treatment for knee osteoarthritis (OA), only symptomatic treatment for pain and loss of function exists[1]. In this context it may be sensible to increase the focus on prevention of the initial development of knee OA[2]. In order to progress in this area we need to detect knee OA in an earlier, preclinical and preradiographic phase.

Currently, no sufficient tools for this aim exist. Plain knee radiography for measuring joint space width has a relatively large precision error and low sensitivity[3]. Magnetic resonance imaging (MRI) is more sensitive in detecting features of knee OA[4], but is not extensively applicable due to costs, long scan time and limited availability[1]. Given the limitations of imaging biomarkers for pre-clinical or pre-radiographic knee OA, biochemical markers are investigated as alternatives[5]. One of these, the Coll2-1NO_2 peptide, represents the combination of collagen type II degradation products (Coll2-1) and reactive nitrogen and oxygen species (RNOS), NO and O_2^-, and can be measured systemically in urine or serum[6]. Elevated production of RNOS has been observed in chronic inflammatory conditions, including established OA, but the effect of the preclinical and preradiographic phase of OA is still unknown[7]. As a low grade chronic inflammation has been suggested to be involved in the development of OA , before visible cartilage degeneration has occurred[8], we might hypothesize that elevated RNOS levels and thus elevated Coll2-1NO_2 concentrations could be measured in the pre-OA phase as well.

The aim of this study is therefore to explore the potency of Coll2-1NO_2 in detecting disease activity in preclinical and preradiographic knee OA, as earlier diagnosis of disease activity enables development of preventive therapies. We explored whether the baseline uColl2-1NO_2 concentration in subjects at risk for developing knee OA was associated with incident knee OA 2.5 years later. Additionally, we explored whether the concentration at 2.5 years was cross-sectionally associated and whether the change in concentration over 2.5 years was associated with incident knee OA.

Method

Study design, setting, and population

We used data from the PROOF study (Prevention of knee Osteoarthritis in Overweight Females, ISRCTN 42823086)[9]. The PROOF study is a randomized controlled trial, with a 2x2 factorial design and 2.5 years follow-up, which evaluates the preventive effects of a diet and exercise program (DEP) and of oral glucosamine sulphate, double blind and placebo controlled (GSvP), on the development of knee OA in overweight and obese middle-aged women. Inclusion criteria were age 50-60 years and BMI ≥ 27 kg/m^2 , as those are proven risk factors for knee OA[10, 11]. All participants were recruited by their General Practitioner (GP) and had to be free of knee OA according to the clinical and radiographic criteria of the American College of Rheumatology (ACR)[12]. The participants had to master the Dutch language and had to be free of major co-morbidities, free of inflammatory rheumatic diseases, not under treatment of a physical therapist or GP for knee complaints, not using walking aids and not using oral glucosamine for the last 6 months. We treated data from PROOF as a pre-clinical OA cohort by adjusting analyses for the randomization groups. The Medical Ethics committee of Erasmus MC University Medical Center Rotterdam approved the PROOF study and all the participants gave written informed consent.

Radiography

Posterior-anterior radiographs of both knees were taken at baseline and at 2.5 years, using the semi-flexed MTP view[13]. A trained researcher blinded for clinical outcomes (MR) scored all radiographs, baseline and follow-up at once with known sequence using the Kellgren & Lawrence (K&L) criteria[14]. A random subset of 20% of the radiographs was independently scored by a second researcher (JR) blinded for clinical outcomes. The Cohen's kappa measure of agreement was moderate with a value of 0.6. Minimal joint space width was measured digitally in each tibiofemoral compartment, according to the method of Lequesne[15], using the average independent score of two researchers (JR and BdV), blinded for the clinical outcomes. Scores with a difference ≥ 2.0 mm between the researchers were re-evaluated in a consensus meeting. The inter-observer agreement for medial and lateral joint space narrowing was substantial with kappa values of 0.67 and 0.76, respectively. Medial anatomical knee alignment angle was assessed on knee radiographs as described previously[16]. Normal alignment was defined as angles between 182° and 184°, valgus and varus alignment were defined as angles > 184° and <182° respectively[17]. The test for reproducibility showed good agreement for alignment with kappa of 0.7[16].

Assessment of Coll2-1NO$_2$

uColl2-1NO$_2$ was determined at baseline and at 2.5 years in non-fasted, second morning void urine samples. The assessment in urine was based upon the qualification of the biomarker according to the BIPED classification: Coll2-1NO$_2$ in urine is qualified as biomarker of prognosis[18]. A detailed description of the identification of Coll2-1NO$_2$ can be found in previous publications[18, 19]. In short, uColl2-1NO$_2$ concentration was assessed by enzyme-linked immunosorbent assay (ELISA) based on the method described by Rosenquist et al[20] using a polyclonal antibody against antigenic determinants of uColl2-1NO$_2$ according to the instructions of the manufacturer (Artialis s.a, Liège, Belgium). 150 µl of urine was needed for each sample. After thawing, total assay time was within a maximum of 3 hours. The precision of the immunoassay of Coll2-1NO2 in urine was previously established by Deberg et al[18] and demonstrated an intra-assay coefficient of variation (CV) of 8.3% and an inter-assay CV of 13.6%. In our study, uColl2-1NO$_2$ was measured in triplicate and two additional urine samples were added on each plate as control. The inter-assay CVs for these two controls were respectively 9.6 and 11%.

uColl2-1NO$_2$ concentration was adjusted for urinary creatinine concentrations by expressing the results as nmol/mmol (nM/mM) creatinine. The creatinine was measured by the method of Jaffé[21] with the MicroVue Creatinine Assay Kit (Quidel, San Diego USA) on a MEGA autoanalyzer (Merck, Germany).

Questionnaires, physical examination and blood samples

At baseline all subjects filled in a questionnaire to record demographic (age, BMI, postmenopausal status, ethnicity) and clinical characteristics including questions on injury, physical activity (measured with the Short QUestionnaire to ASsess Health-enhancing physical activity (SQUASH)[22]), knee complaints ("did you experience knee pain in the past 12 months?") and 'self-reported' OA in other joints. Body weight, body height, blood pressure, abdominal circumference, skin folds and Heberden's nodes on both hands were assessed at the research center. Non-fasted HbA1c concentration (mmol/mol) and total cholesterol concentration (mmol/L) were determined from blood samples taken at baseline.

Outcome

The primary outcome measure of this study was incidence of knee OA in one or both knees at 2.5 years. Incidence of knee OA was defined as either Kellgren & Lawrence (K&L) grade ≥ 2, joint space narrowing (JSN) of ≥ 1.0mm[23] or knee OA according to the combined clinical and radiographic ACR criteria (ACR knee OA). Secondary outcome measures were the separate clinical and radiographic definitions of the primary outcome.

Statistical analysis

For the present study, participants with available baseline and 2.5 years uColl2-1NO_2 concentrations and with a complete follow-up were included for analysis. Baseline characteristics were described as percentages for categorical/dichotomous data and as means ± standard deviation (SD) or medians (interquartile range, IQR) for continuous data. For exploratory analyses, we conducted paired and independent-samples Student's t-tests with untransformed uColl2-1NO_2 data; The paired t-test to evaluate the difference between mean uColl2-1NO_2 at baseline and 2.5 years within the incident and non-incident knee OA women; The independent-samples t-tests to compare baseline-, 2.5 years- and change over 2.5 years- concentrations between the women with and without incident knee OA.

For the regression analyses of uColl2-1NO_2 with primary and secondary outcomes, uColl2-1NO_2 was logarithmically transformed to obtain normally distributed residuals. First, possible confounding variables and prognostic factors in the association of uColl2-1NO_2 with the primary and secondary outcomes were determined by univariable linear regression analyses. The selection of the different demographic, metabolic, functional and radiographic variables was based on their possible relation with uColl2-1NO_2 and knee OA[10, 19, 24]. Variables with a univariable p-value < 0.2 and with an r-value < 0.7 (cut-off point for multicollinearity) were adopted in a multivariable regression analysis (using the Enter method) to analyse significant associations with uColl2-1NO_2.

Subsequently, we analysed the association of uColl2-1NO_2 with the primary and secondary outcome measures. First, we determined the association of baseline uColl2-1NO_2 through binary logistic regression, using 3 different models. The first model was unadjusted, the second model was adjusted for age and BMI, as these are established risk factors for knee OA. The fully adjusted model 3 was adjusted for age, BMI, randomization groups (DEP, GSvP and their multiplicative interaction), possible confounders and prognostic factors from the multivariable analysis and for K&L grade at baseline (0 versus 1), as this has already been shown to be a prognostic factor for incident knee OA in the PROOF study[25]. Next, we analysed the cross-sectional associations of uColl2-1NO_2 with prevalent knee OA and secondary outcomes at 2.5 years to evaluate the diagnostic value of uColl2-1NO_2 . Finally, we analysed the association of the change in uColl2-1NO_2 concentration over 2.5 years, corrected for baseline concentration, with the primary and secondary outcomes. All analyses were performed with the three models.

To facilitate interpretation of the regression associations, uColl2-1NO2 was standardized into z-scores. Results for the regression analyses were presented as odds ratios per standard deviation (SD) increase in log uColl2-1NO_2 and their corresponding 95% confidence intervals. Statistical analyses were performed with SPSS 20.0 (Chicago, IL). A p-value < 0.05 was defined as statistically significant.

Results

Characteristics of the study population

254 of 407 women with mean age of 55.8 years ± 3.19 and mean BMI of 31.0 kg/m^2 ± 3.97 were available for current analyses. The reasons for missing data were as follows: 1) unwilling to continue participation (28/407), 2) unattainable during follow-up (12/407), 3) no urine to the lab (8/407) 4) sample below the limit of detection of the test (61/407), 5) excluded based on K&L >=2 at baseline (42/407) and 6) deceased during follow-up (2/407). Analysis of the baseline differences between missing and non-missing subjects showed a statistically significant higher fat percentage (44.4% vs 43.0%), lower cholesterol concentration (5.9mmol/L vs 6.1mmol/L) and a higher percentage of varus alignment (55.7% vs 44.8%) in those missing. These differences did not seem to be relevant, as no correlation of these variables with Coll2-1NO_2 was found. Distribution, means and/or medians of baseline characteristics are displayed in table 1.

Incident knee OA according to the primary outcome was found in 72/254 women (28.3%). Medial joint space narrowing (JSN) was found in 27/254 (10.6%), lateral JSN in 26/254 (10.2%), ACR defined knee OA in 20/254 (7,9%) and K&L grade ≥ 2 in 23/254 women (9.1%).

Exploratory associations between uColl2-1NO_2 and incident knee OA

Mean uColl2-1NO_2 concentration for the total study group was 0.033nM/mM creatinine ± 0.017 at baseline and 0.034nM/mM ± 0.017 at 2.5 years. The mean creatinine value of all samples was 7.69mM/L ± 4.36. Mean baseline uColl2-1NO_2 concentration was significantly lower in the women with incident knee OA as primary outcome after 2.5 years compared to the women without incident knee OA (0.029nM/mM ± 0.013 versus 0.034nM/mM ± 0.017, p = 0.03). The concentration at 2.5 years showed no significant difference between the women with and without incident knee OA (0.034nM/mM ± 0.018 versus 0.034nM/mM ± 0.017, p = 0.76). Although the change from baseline over 2.5 years within both groups was not significant, the change between both groups was. The mean increase in the women with incident knee OA was 0.005nM/mM ± 0.021 versus a mean decrease of 0.001nM/mM ± 0.020 in the women without incident knee OA (p = 0.04), see figure 1.

Baseline associations between uColl2-1NO_2 and incident knee OA

The variables ethnicity (Caucasian), weight, Heberden's nodes, SQUASH score and 'self-reported' OA in other joints were positively associated with uColl2-1NO_2. Age and years since menopause were negatively associated with uColl2-1NO_2. The variables BMI, waist circumference, fat percentage, total cholesterol, HbA1c, K&L grade 0 vs 1, knee alignment, mild knee symptoms and history of knee injury were not univariable associated with uColl2-1NO_2. In the multivariable regression analyses, none of the variables were significantly associated with uColl2-1NO_2.

The associations of baseline uColl2-1NO_2 with primary and secondary outcomes are displayed in table 2, showing a significant inversed association between baseline uColl2-1NO_2 and incident knee OA at 2.5 years, both in adjusted model 2 and 3 (OR 0.74, 95% CI 0.55-0.99 in model 3). No significant associations were found for the secondary outcomes.

Associations of uColl2-1NO_2 at 2.5 years and prevalent knee OA

The uColl2-1NO_2 concentration at 2.5 years did not show a significant cross-sectional association with prevalent knee OA (OR 1.03, 95% CI 0.77 – 1.37 in model 3) or with the separate outcome definitions, in any of the models (medial JSN: OR 0.93, 95% CI 0.63 – 1.38, lateral JSN: OR 0.88, 95% CI 0.57 – 1.34 , ACR knee OA: OR 1.39, 95% CI 0.82 – 2.37, and K&L ≥ 2: OR 0.92, 95% CI 0.57 – 1.47, all in model 3).

Change of uColl2-1NO_2 and incident knee OA

No significant association was found between the change in concentration over 2.5 years and incident knee OA (OR 1.10, 95% CI 0.81 – 1.48 in model 3), nor for the association with the separate outcome definitions, in any of the models (medial JSN: OR 0.94, 95% CI 0.62 – 1.41, lateral JSN: OR 0.88, 95% CI 0.57 – 1.36, ACR knee OA: OR 1.55, 95% CI 0.88 – 2.72, and K&L ≥ 2: OR 0.97, 95% CI 0.60 – 1.57, all in model 3).

Discussion

This is the first study that assessed the uColl2-1NO_2 biomarker in a high-risk pre-OA cohort of middle-aged overweight and obese women. We found that a lower baseline uColl2-1NO_2 concentration was significantly associated with an increased risk of incident knee OA after 2.5 years. The cross-sectional association between uColl2-1NO_2 at 2.5 years and prevalent knee OA and the association between the change of uColl2-1NO_2 and incident knee OA were not statistically significant.

Context

Serum Coll2-1NO_2 was found to be significantly elevated in knee OA patients, compared to age-matched controls[19]. In another knee study, the one year uColl2-1NO_2 change from baseline, was shown to be predictive for radiographic medial joint space narrowing over 3 years[18]. Our study, unlike the others, was performed with patients at risk for knee OA instead of established knee OA.

Against our expectations, a lower baseline uColl2-1NO_2 concentration was found in the women who developed incident knee OA, compared to those who did not. In vitro studies[26-28] indicate that in the development of OA, besides catabolic inflammatory processes, compensatory anti-inflammatory mechanisms occur in an attempt by chondrocytes to restore cartilage homeostasis[27]. In vitro studies show that anti-inflammatory cytokine IL-10 can inhibit NO expression[28] and can antagonize chondrocyte apoptosis[26]. These studies might give some support for our, somewhat counterintuitive finding of lower baseline uColl2-1NO_2 formation. However, we can only speculate on the role of anti-inflammatory mechanisms, as this had not been studied comprehensively so far in the context of OA[29]. Moreover, some studies suggest that the anti-inflammatory response may never control the inflammatory response in OA completely[30]. We do not know how this balance is acting in the preclinical and preradiographic phase as studied in the present study. Besides, we might also hypothesize that subjects who develop OA have initially lower amounts of cartilage, which reduce the overall formation of uColl2-1NO_2.

We did perform our analyses on person level instead of knee level for different reasons. First, we had the aim to analyse the associations for women and not for knees. The biomarker was furthermore measured systemically and not locally. Moreover, a total of 72 women developed knee OA after 2.5 years follow-up, but only 14 of them had bilateral knee OA. As a result, this would not provide enough power to distinguish between uni- and bilateral knee OA. In ordinal regression analyses (data not shown) we found stronger, but not significant, associations for bilateral compared to unilateral knee OA.

In our exploratory analyses, we found a significant difference in change of uColl2-1NO_2 concentration over 2.5 years between incident and non-incident knee OA. Previously, Deberg et al suggested that uColl2-1NO_2 levels do not increase in preclinical and preradiographic OA phase, but later in OA development[18]. This is supported by the significant increase of uColl2-1NO_2 in women with incident knee OA compared to the women without knee OA development. This increase of uColl2-1NO_2 over time might be caused by the eventual failure of the above mentioned compensatory anti-inflammatory mechanisms during further development of knee OA. However, the significance is found only in our exploratory non-logarithmically transformed analyses.

In the 2.5 years cross-sectional data and in the change of uColl2-1NO_2 concentration over 2.5 years, the positive association with ACR knee OA was most pronounced, albeit not statistically significant. The absence of significance might be due to the small number of women who developed ACR knee OA (20/254, 7.9%) or the relatively short follow-up period of 2.5 years. The relation between (chronic) inflammation and knee pain[31, 32] and between (chronic) inflammation and osteophytes[33] as described in literature, seems to be reflected by this finding of a positive trend for the association between uColl2-1NO_2 (inflammatory marker) and ACR knee OA (pain and osteophytes).

Strengths and limitations

The major strength of this study is its focus on preclinical and preradiographic knee OA. Especially in high risk subjects there is a need for tools that could help detecting disease activity in this phase of knee OA. The assessment of the potency of the uColl2-1NO_2 biomarker in this study is contributing to fulfil this need.

We are aware of the relatively high number of analyses performed, resulting in an increased risk of a type I error. Nevertheless, given the exploratory nature of this study, these results should be seen as the first step in the validation of the uColl2-1NO_2 biomarker in high-risk pre-OA women.

One of the limitations of this study is that we could not undoubtedly exclude the presence of OA in other joints than the knee, which might have influenced the level of systemic uColl2-1NO_2. However, we have taken the presence of Heberden's nodes and the self-reported OA in other joints into account in our analyses. Choosing for self-reported OA is used in more studies[34, 35]. Moreover, the participants in the present study were asked to identify the location of their OA from a list of five (hip, ankle, hand, back/neck, other), which is known to improve the accuracy of self-reporting[36]. In this way we intended to correct as precisely as possible, making the results applicable to the knee joints.

Conclusions and implications

In this study of overweight and obese middle-aged women at risk for developing knee OA, lower baseline uColl2-1NO_2 levels were significantly associated with increased risk of overall incidence of knee OA 2.5 years later. These results might be caused by compensatory mechanisms in the preclinical and preradiographic phase of the pathophysiologic process, lower NO production or an overall lower cartilage volume in people developing knee OA.

In the preclinical and preradiographic phase, distinguishing subjects who are at risk to develop definite knee OA from those who are not, has a high priority. It seems important to further validate the Coll2-1NO_2 biomarker and to increase our understanding of this very early phase of knee OA to enable development of preventive therapies for those subjects prone to develop knee OA.

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Author contributions

ML contributed to the analysis and interpretation of data, writing of the manuscript and final approval of the article.

JR contributed to the conception and design of the study including collection and assembly of data, analysis and interpretation of data and critical revision of the article for important intellectual content.

SBZ contributed to conception and design of the study including obtaining of funding, analysis and interpretation of data and critical revision of the article for important intellectual content.

YH contributed to the laboratory work, to the interpretation of data and to the critical revision of the article for important intellectual content.

MvM, GvO, BK, PB, EO, DV, MR contributed to the conception and design of the study and to the critical revision of the article for important intellectual content.

All authors approved the final version of the manuscript.

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Competing interest statement

Y Henrotin is the founder and chairman of the university spin-off Artialis sa.

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Table 1. Mean (± SD) or median (IQR) of baseline variables.

SD = standard deviation. IQR = interquartile range. * Higher scores represent higher physical activity. ** Higher scores represent more pain/stiffness/worse function. *** JSW: joint space width

N-subjects	**254**
General	
Age (yr)	55.8 ± 3.19
Ethnicity	
Western	95.7%
Other	3.1%
Postmenopausal status	69.7%
Years postmenopausal	7.6 ± 5.3
Metabolic	
BMI (kg/m2)	31.9 ± 3.97
Weight (kg)	87.3 ± 12.7
Physical activity score (SQUASH)*	7058.3 ± 3672.4
Joint specific	
Heberden's nodes	27.2%
WOMAC (0 – 100)**	
Pain	6.2 ± 10.13
Function	6.2 ± 10.13
Stiffness	11.4 ± 17.0
K&L	
grade 0 bilateral	45.3%
grade 1 unilateral	22.4%
grade 1 bilateral	32.3%
Minimal JSW***	
medial (mm)	4.9 ± 0.7
lateral (mm)	6.1 ± 0.9
Varus alignment	
Unilateral	17.7%
Bilateral	26.8%
Mild symptoms	
Unilateral	25.6%
Bilateral	17.3%
History of knee injury	
Unilateral	17.7%
Bilateral	2.8%
Biomarker	
Mean uColl2-1NO_2/creatinine (nM/mM)	0.0330 ± 0.0165
Median uColl2-1NO_2/creatinine (nM/mM)	0.0313 (IQR 0.0220 – 0.0406)

Figures

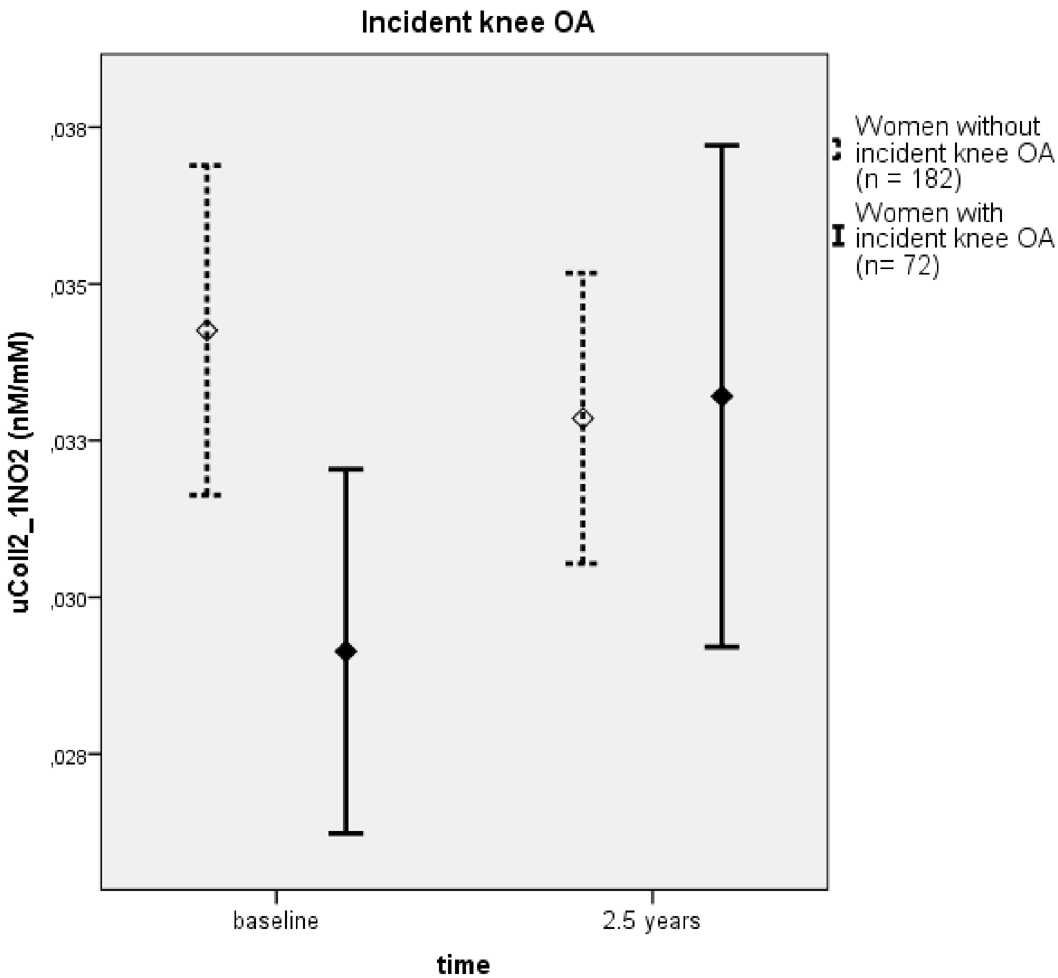

Figure 1: uColl2-1NO_2 (nM/mM) levels at baseline and 2.5 years follow-up for women without and with incident knee OA at 2.5 years, not adjusted for BMI, age, K&L grade (0 vs 1) and randomization groups. P-values obtained from paired t-tests, to evaluate the difference between mean uColl2-1NO_2 at baseline and 2.5 years within the incident and non-incident knee OA women. P-value* is obtained from unpaired t-test, to compare the change over 2.5 years in the women with and without incident knee OA. P-value** is obtained from unpaired t-test, to compare the baseline difference in women with and without incident knee OA.

BL = Baseline, FU = Follow-up

Table 2. Multivariable adjusted association between uColl2-1NO_2 and adjusted variables age, BMI and K&L grade (0 vs 1) at baseline and overall incident knee OA and separate incidence definitions, at 2.5 years.

Bold indicates p-value < 0.05

CI = confidence interval. OA = osteoarthritis

† Incidence of knee OA at 2.5 years: either Kellgren & Lawrence grade ≥ 2, joint space narrowing (JSN) of ≥ 1.0mm or knee OA according to the combined clinical and radiographic ACR criteria

‡ secondary outcomes: separate definitions of incidence of knee OA

* model 1: unadjusted

** model 2: adjusted for age and body mass index

*** model 3: adjusted for age, body mass index, randomisation groups, interaction between randomisation groups and K&L grade (0 vs 1) at baseline

			uColl2-1NO$_2$		Age		BMI		K&L 0 vs 1	
	Cases (%)		**OR**	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**
Incident knee OA†	72/254 (28.3)	Model 1*	0.77	0.58 – 1.02		-		-		-
		Model 2**	**0.74**	**0.56 – 0.99**	1.03	0.94 – 1.22	**1.10**	**1.03 – 1.18**		-
		Model 3***	**0.74**	**0.55 – 0.99**	1.02	0.94 – 1.12	**1.09**	**1.01 – 1.17**	1.77	0.98 – 3.20
Medial JSN‡	27/254 (10.6)	Model 1*	0.99	0.65 – 1.49		-		-		-
		Model 2**	0.83	0.63 - 1.46	0.99	0.87 – 1.13	1.08	0.98 – 1.18		-
		Model 3***	1.00	0.61 - 1.49	1.00	0.88 – 1.13	1.06	0.96 – 1.16	1.37	0.58 – 3.23
Lateral JSN‡	26/254 (10.2)	Model 1*	0.95	0.63 – 1.44		-		-		-
		Model 2**	0.94	0.62 – 1.43	1.02	0.89 – 1.16	1.05	0.95 – 1.16		-
		Model 3***	0.95	0.63 – 1.43	1.02	0.89 – 1.17	1.10	0.99 – 1.22	**0.38**	**0.16 – 0.93**
ACR criteria‡	20/254 (7.9)	Model 1*	0.77	0.51 – 1.18		-		-		-
		Model 2**	0.72	0.47 – 1.12	0.96	0.83 – 1.12	**1.11**	**1.00 – 1.23**		-
		Model 3***	0.70	0.43 - 1.12	0.96	0.83 – 1.12	1.07	0.97 – 1.19	**7.87**	**1.74 – 35.55**
KL ≥ 2‡	23/254 (9.1)	Model 1*	0.83	0.55 – 1.25		-		-		-
		Model 2**	0.78	0.50 – 1.20	1.08	0.93 – 1.25	**1.18**	**1.08 – 1.30**		-
		Model 3***	0.74	0.47 - 1.18	1.07	0.93 – 1.24	**1.15**	**1.04 – 1.26**	**3.44**	**1.09 – 10.85**