Osteoarthritis and Cartilage 23 (2015) 397–404

Osteoarthritis and Cartilage

OARSI OSTEOARTHRITIS RESEARCH SOCIETY INTERNATIONAL

Association of adipokines and joint biomarkers with cartilage-modifying effects of weight loss in obese subjects

L.K. King †, H. Henneicke ‡, M.J. Seibel ‡, L. March § ||, A. Anandacoomarasmy † ¶ *

† *Sydney Medical School, The University of Sydney, Australia*
‡ *Bone Research Program, ANZAC Research Institute, The University of Sydney, Australia*
§ *Institute of Bone and Joint Research, Kolling Institute of Medical Research, The University of Sydney, Australia*
|| *Department of Rheumatology, Royal North Shore Hospital, Sydney, Australia*
¶ *Department of Rheumatology, Concord Hospital, Sydney, Australia*

ARTICLE INFO

Article history:
Received 1 May 2014
Accepted 25 November 2014

Keywords:
Obesity
Knee osteoarthritis
Cartilage
Biomarkers
Adipokines

SUMMARY

Objectives: To determine (1) the effects of weight loss in obese subjects on six adipokines and joint biomarkers; and (2) the relationship between changes in these markers with changes in cartilage outcomes.
Design: Plasma levels of adiponectin, leptin, IL-6, COMP, MMP-3 and urine levels of CTX-II were measured at baseline and 12 months from 75 obese subjects enrolled in two weight-loss programs. Magnetic resonance imaging (MRI) was used to assess cartilage volume and thickness. Associations between weight loss, cartilage outcomes and markers were adjusted for age, gender, baseline BMI, presence of clinical knee OA, with and without weight loss percent.
Results: Mean weight loss was 13.0 ± 9.5%. Greater weight loss percentage was associated with an increase in adiponectin ($\beta = 0.019$, 95% CI 0.012 to 0.026,) and a decrease in leptin ($\beta = -1.09$, 95% CI −1.37 to −0.82). Multiple regression analysis saw an increase in adiponectin associated with reduced loss of medial tibial cartilage volume ($\beta = 14.4$, CI 2.6 to 26.3) and medial femoral cartilage volume ($\beta = 18.1$, 95% CI 4.4 to 31.8). Decrease in leptin was associated with reduced loss of medial femoral volume ($\beta = -4.1$, 95% CI −6.8 to −1.4) and lateral femoral volume ($\beta = -1.8$, 95% CI −3.7 to 0.0). When weight loss percent was included in the model, only the relationships between COMP and cartilage volume remained statistically significant.
Conclusions: Adiponectin and leptin may be associated with cartilage loss. Further work will determine the relative contributions of metabolic and mechanical factors in the obesity-related joint changes.

Introduction

Osteoarthritis (OA), is the clinical syndrome of joint pain and dysfunction caused by joint degeneration[1], and of the weight-bearing joints, the knee is most frequently involved[2]. Obesity is a significant risk factor for both the incidence and progression of knee OA[3–5]. The mechanisms by which obesity contributes to knee joint structural damage are thought to be secondary to both mechanical and metabolic factors[6], as being obese also increases the risk of OA in non-weight-bearing joints such as the hands[7]. The precise metabolic pathways through which obesity contributes to knee joint structural damage are currently not known, although thought to involve aberrant adipokine expression with direct and downstream effects leading to the destruction and remodeling of joint tissue[8,9].

White adipose tissue is now known to be an abundant source of mediators involved in many disease processes[10]. Adipokines exert modulatory actions on target tissues and cells involved in OA, including cartilage, synovium and bone. Leptin and adiponectin are the most abundantly produced adipokines[11] and their receptors are expressed on the surface of chondrocytes, synoviocytes and subchondral osteoblasts[12–14]. Histologically assessed depletion of proteoglycan in articular cartilage was observed after leptin was injected into the knee joint of rats[15]. Leptin may

* Address correspondence and reprint requests to: A. Anandacoomarasmy, University of Sydney, Department of Rheumatology, Concord General Hospital, Concord West, NSW 2139, Australia. Tel: 61-2-9767-6831; Fax: 61-2-9767-5421.
E-mail addresses: laurenkarinaking@gmail.com (L.K. King), holger.henneicke@googlemail.com (H. Henneicke), markus.seibel@sydney.edu.au (M.J. Seibel), lyn.march@sydney.edu.au (L. March), anaananda@med.usyd.edu.au, ana.ananda@sydney.edu.au (A. Anandacoomarasmy).

http://dx.doi.org/10.1016/j.joca.2014.11.020
1063-4584/

produce pro-inflammatory and catabolic effects on cartilage through induction of matrix metalloproteinases (MMPs), nitric oxide and production of pro-inflammatory cytokines[12,16–18]. Levels of adipokines in obese people may be particularly important, as obesity may produce a biochemical environment in which chondrocytes cannot respond to such challenges; chondrocytes from obese OA patients have been shown to exhibit a response pattern to leptin different from normal or overweight patients[19].

Less is known about the role of adiponectin in joint disease, with both pro-inflammatory and anti-inflammatory properties being reported locally[8,20,21], compared to its systemic anti-inflammatory effects[11]. Levels of leptin and adiponectin are significantly elevated in people with OA compared to controls[22]. A recent study of obese subjects with knee OA demonstrated decreased circulating levels of leptin and increased circulating levels of adiponectin after weight loss[23], raising the question of the effects of adipokines on OA disease progression.

Biomarkers offer a promising new approach for early diagnosis and monitoring the progression of OA. As loss of articular cartilage is a hallmark of the disease, biomarkers of cartilage degradation have received the most attention. Serum cartilage oligomeric matrix protein (COMP) and urine c-telopeptide of type II collagen (CTX-II) are both markers of cartilage degradation according to the "BIPED" classification[24], and have had the best performance of all currently available commercial biomarkers[25]. Levels of both have been correlated with cartilage defects on magnetic resonance imaging (MRI) in cross-sectional studies[26–28], although correlation between change in these markers over time and cartilage outcomes are not well established[28,29]. Biomarkers of bone and synovial turnover have also been studied, with less promising results so far[25]. Other studies have investigated MMPs, which are molecules produced from chondrocytes that attack aggrecan, fibronectin and type II collagen. In particular, MMP-3 is elevated in the blood of patients with knee OA[30], and reduction over time has been correlated with reduction in cartilage volume loss[31].

Weight loss in obese subjects has been shown to reduce symptoms of knee OA and reduce future progression of the disease. Recently it was demonstrated that obese people who lose weight have reduced cartilage loss[32]. Previous studies have found a decrease in COMP serum concentrations in obese subjects who lose weight through bariatric surgery[23] or diet and exercise interventions[33]; however, these studies did not find any relationship between COMP and adipokine changes, and did not assess cartilage loss.

Our aim was to investigate the effect of weight loss on adipokines and joint biomarkers in obese subjects with and without clinical knee OA, and to measure for the first time the association between changes in level of markers and changes in cartilage outcomes on MRI in this population.

Method

Participants

Details of this cohort were previously reported in Refs. 32,34. One hundred and eleven patients were prospectively recruited from two weight loss programs (non-surgical and surgical) at two urban tertiary referral hospitals, public and private, respectively. All subjects were obese with the majority being obesity grade II or higher. The non-surgical program involved dietary modification and exercise, and the surgical group underwent laparoscopic adjustable banding; all subjects had self-enrolled. Exclusion criteria included inflammatory arthritis. Seventy-eight subjects completed MRI follow-up for cartilage assessment at 12 months, of whom 75 subjects had sufficient volume of stored plasma and urine samples for adipokine and joint biomarker analysis.

Assessments

Subjects were assessed at recruitment (baseline, *prior to* start of weight-loss program) and again 12 months later.

Clinical assessment

Weight loss, weight loss percent, waist–hip ratio and BMI were assessed. The diagnosis of knee OA was based on the clinical criteria described by Altman *et al.*[35].

Cartilage measurements

Subjects underwent baseline MRI of the symptomatic or dominant asymptomatic knee. Sagittal MRI images were obtained on a 3T scanner (Magnetom Trio; Siemens, Erlangen, Germany) as previously described in Ref. 34. Cartilage volume and thickness were measured by a single trained reader by performing cartilage segmentation (weight-bearing femoral cartilage and all of the tibial cartilage) using propriety software (Chondrometrics, Ainring, Germany), which has been shown to be both reliable and valid[36]. The reader (AA) was blinded to all clinical data. The change in both knee cartilage volume and thickness over 12 months was calculated for the medial tibial, medial femoral, lateral tibial and lateral femoral compartments by subtracting the follow-up value from the baseline value. The intraclass correlation (ICC) was >0.91 for each region of interest.

Biochemical assays

Venous blood and urine were collected at baseline and 12 months in the non-fasted state. The blood was centrifuged to separate plasma, and then the specimens were aliquoted into 1 ml cryotubes. Plasma and urine were stored at −80°C until analyzed. Plasma levels of leptin, adiponectin, interleukin-6 (IL-6), COMP and MMP-3 and urine concentrations of CTX-II were assessed for each sample and time point.

All markers were determined using specific assay kits in accordance with manufacturers' instructions. Quantikine Human ELISA kits (R&D systems, Inc., Minneapolis, Minnesota, USA) were used for the determination of adiponectin (sensitivity 0.25 ng/ml, intra- and inter-assay coefficient of variation (CVs) 6.5% and 3.5%, respectively), leptin (sensitivity 7.8 pg/mL, and intra- and inter-assay CVs 4.4% and 3.2%), respectively, IL-6 (sensitivity 0.7 pg/ml, and intra- and inter-assay CVs 2.7% and 3.1 %, respectively), COMP (sensitivity 0.01 ng/ml, and intra- and inter-assay CVs are 4.6% and 3.2%, respectively) and MMP-3 (sensitivity 0.009 ng/mL, and intra- and inter-assay CVs 7.8% and 6.1%, respectively). The Urine Cartilaps ELISA (IDS, Boldon, United Kingdom) was used for determination of CTX-II (sensitivity 0.20 ng/mL, intra- and inter-assay CVs 4.6% and 8.1%, respectively). Urinary creatinine was measured by a routine chemistry method and used for calculation of creatinine-corrected urinary CTX-II concentrations. Corrected CTX-II value (μg/mmol) was calculated as CartiLaps CTX-II (μg/L)/creatinine (mmol/L).

All samples were measured in duplicate for each marker. The average of the two values was reported and used in data analyses. Duplicate samples that did not provide a CV of <15% as well as sample readings outside of the published assay range were disregarded from the analysis.

Statistical analysis

Descriptive statistics were performed and reported as mean (SD). Paired *t*-test and Wilcoxon matched-pair signed-rank test

were used to compare levels of adipokines and joint biomarkers at baseline and 12 months. Logarithmic transformation was performed for adiponectin, IL-6, OCMP, MMP-3 and CTX-II as values did not follow a normal distribution. Spearman correlation analysis was used to analyze the association between change in each marker and percentage weight loss, as well as with changes in cartilage volume. The Mann–Whitney test was used for categorical variables. Linear regression was used to examine the associations between percentage weight loss and markers, as well as markers and cartilage outcomes. Multiple regression analyses were used to estimate parameters, and were adjusted for age, gender, baseline BMI, presence of clinical knee OA and weight loss percent as these parameters have previously been shown to be associated with change in cartilage parameters. All statistical analyses were performed using SPSS standard version 19.0 (SPSS, Chicago, Illinois, USA). A P value <0.05 was considered statistically significant for all comparisons. Beta (β) was used designate the standardized co-efficients derived from the multiple regression models.

Results

Demographic characteristics of patients

Our study population consisted of 75 obese subjects. Characteristics of this cohort are presented in Table I. Mean weight loss percent at 12 months was 13.0 ± 9.5%. The percentage of subjects who achieved any weight loss at 12 months was 80%. The group that underwent surgery for weight loss achieved greater percentage body weight loss than the non-surgical group (17.0 ± 7.4% and 3.1 ± 6.6%, respectively).

Mean cartilage volume loss over 12 months was 45.9 ± 149 mm^3, 44.1 ± 173 mm^3, 61.9 ± 189 mm^3 and 67.3 ± 114 mm^3 for the medial tibial (MT), medial lateral (MF), lateral tibial (LT) and lateral femoral (LF) compartments, respectively. Mean cartilage thickness loss over 12 months was 0.017 ± 0.127 mm, −0.007 ± 0.223 mm, 0.003 ± 0.140 mm and 0.061 ± 0.174 mm for the MT, MF, LT and LF compartments, respectively.

Effect of time and weight loss on adipokines and joint biomarkers

The levels of adipokines and biomarkers at baseline and 12 months are shown in Table II. As expected, mean levels of adiponectin increased ($P < 0.001$) and mean levels of leptin decreased ($P = 0.03$). IL-6 was found to increase in our subjects ($P < 0.001$). Furthermore, we observed a decrease in COMP ($P = 0.009$) over 12 months but there was no statistically significant change in plasma concentrations of MMP-3 or urine CTX-II.

The association between weight change and adipokines and biomarkers are presented in Table III. Greater weight loss was associated with a statistically significant increase in adiponectin, and a statistically significant decrease in leptin (Fig. 1). It was also associated with a statistically significant decrease in COMP, but there was no statistically significant association with changes in IL-6, MMP-3 or CTX-II concentrations.

Table I
Characteristics of obese cohort

Characteristic	Baseline	12 months	P value
Age, yrs ± SD (range)	52 ± 12 (26–77)	NA	NA
Females, n (%)	52 (69.3)	NA	NA
Clinical knee OA, n (%)	23 (30.7%)	NA	NA
Surgical patients, n (%)	26 (34.6%)	NA	NA
BMI, kg/m^2 ± SD	39.9 ± 5.1	36.6 ± 5.8	<0.001
Weight, kg ± SD	111.8 ± 8.9	102.5 ± 18.3	<0.001
Waist, cm ± SD	119.0 ± 13.0	113.5 ± 14.7	<0.001
Waist–Hip ratio ± SD	0.92 ± 0.09	0.91 ± 0.08	<0.001

OA, osteoarthritis; BMI, body mass index.

Table II
Levels of adipokines and biomarkers at baseline and 12 months

	Baseline		12 Months		P^* value
	No.	Median (interquartile range)/mean (SD)	No.	Median (interquartile range)/mean (SD)	
Adiponectin, μg/ml	72	4.8 (3.0–6.4)	74	6.6 (4.1–9.9)	<0.001
Leptin, ng/ml	74	44.8 (20.0)	74	41.1 (24.4)	0.03
IL-6, pg/ml	50	2.6 (1.7–4.3)	70	3.8 (2.6–5.5)	<0.001
COMP, ng/ml	74	233 (167–327)	74	203 (149–299)	0.009
MMP-3, ng/ml	74	5.7 (3.9–10.0)	74	5.1 (3.6–8.8)	0.12
uCTX-II, μg/mmol	71	268 (173–359)	64	308 (175–463)	0.20

*Wilcoxon marched-pair signed-rank test or Paired t-test where appropriate.

Effect of other variables on adipokines and joint biomarkers

There was no statistically significant effect of age, gender or clinical knee OA on changes in adipokines and/or joint biomarkers.

Effect of levels of adipokines and biomarkers on cartilage outcomes

Cartilage outcomes are presented in Table IV.

For cartilage volume, in multiple regression analysis adjusted for age, baseline BMI, gender and clinical knee OA, an increase in adiponectin was associated with reduced loss of MT cartilage volume ($P = 0.02$) and MF cartilage volume ($P = 0.01$). A decrease in leptin was associated with reduced loss of MF ($P = 0.004$) and LF cartilage volume ($P = 0.05$). A decrease in COMP was associated with reduced loss of MT ($P = 0.007$) and LT cartilage volume ($P = 0.03$). When weight-loss percent was added to the model, the association between reduced COMP and reduced MT and LT volume loss remained statistically significant.

For cartilage thickness, in multiple regression analysis adjusted for age, baseline BMI, gender and clinical knee OA, an increase in adiponectin was associated with reduced loss in MT thickness ($P = 0.03$). A decrease in leptin was associated with reduced MF cartilage thickness loss ($P = 0.02$) and a decrease in COMP was associated with reduced MT cartilage thickness loss ($P = 0.007$). When weight loss percent was included in the model the association between reduced COMP and reduced MT cartilage loss remained statistically significant ($P = 0.01$).

In multiple regression analysis adjusted for age, baseline BMI, gender and clinical knee OA, baseline or change in leptin-to-adiponectin ratio was not associated with either baseline or change in any of the MRI cartilage outcomes, respectively.

There was no statistically significant association observed between changes in adipokines and joint biomarkers with changes in lateral cartilage volume or thickness. Baseline levels of adipokines and joint biomarkers were not prognostic of cartilage changes over the 12 months study period. Waist/hip ratio did not affect cartilage parameters.

Discussion

This study provides evidence that appropriate plasma levels of adiponectin and leptin may be associated with a preservation of

Table III
Associations between weight change and adipokines and biomarkers at 12 months

Regression models		Coefficient* (95% CI)	*P* value	Coefficient† (95% CI)	*P* value
Dependent variable	Independent variable				
Adiponectin	Weight loss (%)	0.019 (0.012, 0.026)	<0.001	0.019 (0.011, 0.026)	<0.001
	Weight loss (kg)	0.014 (0.008, 0.020)	<0.001	0.015 (0.009, 0.021)	<0.001
Leptin	Weight loss (%)	−1.09 (−1.37, −0.82)	<0.001	−1.13 (−1.41, −0.84)	<0.001
	Weight loss (kg)	−0.83 (−1.06, −0.61)	<0.001	−0.87 (−1.11, −0.63)	<0.001
IL-6	Weight loss (%)	−0.001 (−0.024, 0.006)	0.23	−0.013 (−0.028, 0.003)	0.10
	Weight loss (kg)	−0.005 (−0.016, 0.007)	0.42	−0.009 (−0.021, 0.003)	0.15
COMP	Weight loss (%)	−0.009 (−0.015, −0.002)	0.01	−0.009 (−0.016, −0.002)	0.01
	Weight loss (kg)	−0.007 (−0.012, −0.001)	0.01	−0.007 (−0.013, −0.002)	0.01
MMP-3	Weight loss (%)	−0.003 (−0.012, 0.005)	0.40	−0.004 (−0.012, 0.004)	0.29
	Weight loss (kg)	−0.002 (−0.008, 0.005)	0.63	−0.004 (−0.010, 0.003)	0.23
uCTX-II	Weight loss (%)	0.004 (−0.013, 0.021)	0.66	0.002 (−0.016, 0.019)	0.84
	Weight loss (kg)	0.004 (−0.010, 0.018)	0.59	0.002 (−0.013, 0.016)	0.80

* Adjusted for baseline value.
† Adjusted for baseline value, age at baseline, gender, baseline BMI and presence of clinical knee OA.

cartilage in (obese) patients with and without knee OA. Obese patients who lost a moderate percentage of their body weight (13%) displayed statistically significantly change in the levels of these circulating adipokines, and these biochemical changes were associated with a reduction in cartilage loss assessed by MRI. This effect may be one of the ways that obesity influences radiological outcomes, although these results suggest that mechanical loading may be the dominant etiology. To our knowledge, there have been no previous studies investigating the effect of weight loss on relationships between adipokines, joint biomarkers and cartilage outcomes on MRI in people with obesity.

In our study, weight loss resulted in a statistically significant increase in adiponectin and decrease in leptin and COMP plasma concentrations, similar to results observed in previous trials[23,33]. A recent study by Richette *et al.* found that in a cohort of patients with obesity and knee OA, surgically induced weight loss (mean: 20%) resulted in a significant increase in adiponectin, decrease in leptin and decrease in COMP, but no relationships between adipokines and joint biomarkers were found[23]. In contrast, in this study we observed a statically significant association between reduction in leptin and reduction in COMP plasma concentrations, independent of weight loss percentage. However, the association between leptin and cartilage loss did not reach statistical significance when weight loss percent was controlled for; this likely reflects the strong correlation between weight loss and changes in leptin as well as the importance of the benefits to articular

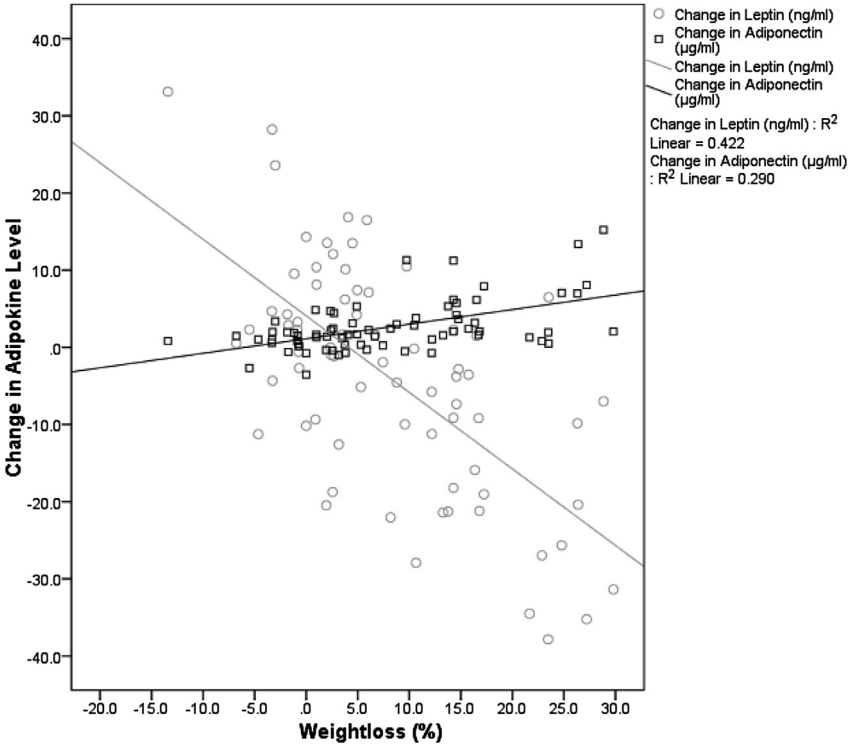

Fig. 1. Association between weight loss (%) and change in adipokines.

Table IV
Associations between changes in adipokines and biomarkers and cartilage outcomes at 12 months

Regression models:		Coefficient* (95% CI)	*P* value	Coefficient† (95% CI)	*P* value
Dependent variable	Independent variable				
MT volume	Adiponectin	14.4 (2.6, 26.3)	0.02	11.1 (−2.5, 24.7)	0.11
	Leptin	−2.0 (−4.5, 0.5)	0.11	−0.7 (−3.9, 2.5)	0.67
	IL-6	12.1 (−8.2, 32.4)	0.24	12.8 (−7.7, 33.3)	0.21
	COMP‡	−7.1 (−12.2, −2.1)	0.007	−6.2 (−11.4, −1.0)	0.02
	MMP-3	−10.2 (−27.5, 7.0)	0.24	−9.5 (−26.4, 7.4)	0.26
	uCTX-II§	−1.0 (−3.0, 0.9)	0.30	−0.9 (−2.8, 1.1)	0.37
MF volume	Adiponectin	18.1 (4.4, 31.8)	0.01	7.1 (−7.7, 21.9)	0.34
	Leptin	−4.1 (−6.8, −1.4)	0.004	−1.3 (−4.7, 2.1)	0.45
	IL-6	−5.9 (−26.1, 14.3)	0.56	−3.7 (−23.7, 16.2)	0.71
	COMP‡	−5.4 (−11.4, 0.7)	0.08	−2.5 (−8.3, 3.3)	0.39
	MMP-3	−6.5 (−26.4, 13.4)	0.52	−4.5 (−22.5, 13.5)	0.62
	uCTX-II§	−0.4 (−2.7, 1.9)	0.72	0.0 (−2.1, 2.1)	1.00
LT volume	Adiponectin	−2.6 (−20.7, 15.6)	0.78	−7.5 (−27.5, 12.5)	0.45
	Leptin	−0.5 (−4.0, 2.9)	0.75	0.9 (−3.8, 5.5)	0.71
	IL-6	10.2 (−10.8, 31.1)	0.33	12.0 (−9.8, 33.7)	0.27
	COMP‡	−7.3 (−14.1, −0.6)	0.03	−7.0 (−13.9, −0.2)	0.04
	MMP-3	−7.7 (−29.6, 14.3)	0.49	−7.1 (−29.2, 14.9)	0.52
	uCTX-II§	−0.4 (−2.8, 2.1)	0.77	−0.2 (2.7, 2.2)	0.86
LF volume	Adiponectin	0.6 (−7.9, 9.1)	0.89	−4.5 (−14.4, 5.5)	0.38
	Leptin	−1.8 (−3.7, 0.0)	0.05	−1.5 (−3.9, 0.9)	0.22
	IL-6	−4.5 (−19.5, 10.5)	0.55	−2.4 (−17.5, 12.7)	0.75
	COMP‡	−2.6 (−6.5, 1.2)	0.18	−2.2 (−6.1, 1.7)	0.27
	MMP-3	−6.0 (−18.6, 6.6)	0.34	−5.2 (−17.7, 7.4)	0.41
	uCTX-II§	−0.6 (−2.0, 0.8)	0.39	−0.5 (−1.9, 0.9)	0.49
MT thickness	Adiponectin	0.011 (0.001, 0.022)	0.03	0.010 (−0.002, 0.022)	0.10
	Leptin	−0.001 (−0.003, 0.001)	0.45	0.000 (−0.003, 0.003)	0.86
	IL-6	0.008 (−0.011, 0.027)	0.40	0.009 (−0.010, 0.028)	0.36
	COMP‡	−0.006 (−0.011, −0.002)	0.007	−0.006 (−0.010, −0.001)	0.01
	MMP-3	−0.003 (−0.018, 0.012)	0.65	−0.003 (−0.018, 0.012)	0.69
	uCTX-II§	−0.001 (−0.002, 0.001)	0.46	−0.001 (−0.002, 0.001)	0.55
MF thickness	Adiponectin	0.017 (−0.001, 0.035)	0.06	0.007 (−0.012, 0.027)	0.46
	Leptin	−0.004 (−0.008, −0.001)	0.02	−0.002 (−0.006, 0.003)	0.44
	IL-6	−0.016 (−0.042. 0.011)	0.24	−0.014 (−0.041, 0.012)	0.28
	COMP‡	−0.003 (−0.011, 0.004)	0.38	−0.001 (−0.009, 0.007)	0.81
	MMP-3	−0.006 (−0.031, 0.020)	0.66	−0.004 (−0.028, 0.020)	0.76
	uCTX-II§	−0.001 (−0.004, 0.002)	0.65	0.000 (−0.003, 0.002)	0.82
LT thickness	Adiponectin	−0.004 (−0.018, 0.009)	0.52	−0.006 (−0.021, 0.009)	0.44
	Leptin	0.000 (−0.003, 0.002)	0.74	−0.001 (−0.004, 0.003)	0.75
	IL-6	−0.004 (−0.018, 0.010)	0.59	−0.004 (−0.019, 0.011)	0.58
	COMP‡	−0.003 (−0.009, 0.002)	0.20	−0.003 (−0.009, 0.002)	0.21
	MMP-3	−0.002 (−0.019, 0.014)	0.80	−0.002 (−0.019, 0.015)	0.80
	uCTX-II§	0.001 (0.001, 0.002)	0.50	0.001 (−0.001, 0.002)	0.51
LF thickness	Adiponectin	−0.002 (−0.016, 0.011)	0.74	−0.003 (−0.020, 0.013)	0.69
	Leptin	−0.002 (−0.005, 0.001)	0.26	−0.003 (−0.007, 0.001)	0.13
	IL-6	−0.009 (−0.033, 0.015)	0.45	−0.009 (−0.033, 0.016)	0.48
	COMP‡	−0.001 (−0.007, 0.006)	0.81	−0.001 (−0.007, 0.006)	0.80
	MMP-3	−0.002 (−0.021, 0.018)	0.88	−0.002 (−0.022, 0.019)	0.88
	uCTX-II§	−0.001 (−0.003, 0.002)	0.57	−0.001 (−0.003, 0.002)	0.58

* Adjusted for baseline value, age at baseline, gender, baseline BMI and presence of clinical knee OA.
† Adjusted for baseline value, age at baseline, gender, baseline BMI, presence of clinical knee OA and weight loss percentage.
‡ Every 10 ng/ml.
§ Every 10 μg/mmol.

cartilage imparted by the reduction in mechanical loading associated with weight loss.

There has been a growing interest in the correlation of OA biomarkers with cartilage outcomes, but to date most evidence is derived from cross-sectional or longitudinal studies with radiographic outcomes rather than MRI. This study therefore provides evidence that changes in circulating adipokines are not only associated with changes in joint biomarkers (here COMP) but may be associated with a preservation of knee cartilage as determined by MRI. Furthermore, we found reduced loss of cartilage was associated with reduction in plasma levels of COMP, adding to the pool of evidence that COMP is a promising biomarker for OA. In accordance with our findings, Berry *et al.* reported that in a group of subjects with knee OA followed for 2 years, changes in levels of COMP correlated with medial cartilage volume loss in subjects with lower-than-average COMP levels[29]. In a recent study by Erhart-Hledik *et al.*, changes in serum COMP observed after a 30-min walk were correlated with cartilage thickness in the medial femur and tibia over a 5-year period indicating that the mechanical stimulus could better delineate cartilage undergoing increased degradation[37].

Studies that have investigated the effect of weight loss on OA biomarkers are scarce and have not studied a general obese population but rather focused on subjects with established, generally

severe, OA[23,33]. This is a marked difference to the present study, in which subjects were recruited from two weight loss programs and only 30% were found to have clinical evidence of OA at baseline. We found no change in MMP-3 or urine CTX-II with moderate weight loss, and no relationship between these markers and cartilage outcomes. Bruyere *et al.* found that an increase in CTX-II after 3 months was significantly correlated with low cartilage thickness at 1 year in a cohort of subjects with established disease[28]. Some authors have suggested that urine CTX-II measurements may not be suitable to detect subtle changes of cartilage metabolism in a single joint indicative of early OA, as uCTX-II release from articular cartilage has to be sufficiently elevated to bio-concentrate in urine, a state more resembling an established OA pathology[38]. Similarly, while MMP-3 has been shown to be elevated in patients with knee OA and predict radiographic progression[30], it may be that MMP-3 was not sufficiently elevated in our cohort to produce detectable change in levels in early disease. Additionally, the short follow-up time may have limited the observation of changes.

Obesity is characterized by a low-grade inflammatory state, leading to its effects on many organ systems. Previous studies have found that weight loss is associated with a decrease in circulating markers of inflammation, such as CRP, TNF and IL-6[23,39–41]. We were surprised not to find this association between weight loss and IL-6. In the present study we did not observe an association between baseline levels of IL-6 and the presence of clinical knee OA, or change in IL-6 and cartilage outcomes; as previously reported associations have been with X-ray changes[42,43]. Recent work has suggested the infra-patellar fat pad may be a more important source of locally active IL-6[44] affecting the knee joint rather than the systemic levels measured in our study.

While a recent study by Hunter *et al.* with similar numbers of patients[45], did not observe an association between weight loss and cartilage changes on MRI, the authors found a trend towards improvements in bone marrow lesions (BMLs). Given the association with BMLs and pain[46], this may be of particular clinical relevance. The sequences to assess BMLs could not be incorporated into our study protocol due to image acquisition restrictions. In this cohort there was no association between adipokines or joint biomarkers and cartilage loss in the lateral compartment, consistent with previous studies[29]. While a systemic process would be expected to affect all compartments of the knee equally, interactions between mechanical and metabolic processes could explain why we observed more effects in the medial compartment. In most subjects in this cohort we found varus alignment, where mechanical loading disproportionally affects the medial compartment of the knee.

This was a prospective cohort study involving a heterogeneous sample of obese subjects, with subjects recruited from both non-surgical and surgical centers. These data have generated hypotheses that serve as a basis for further study. MRI imaging at the knee has also allowed us to measure cartilage volume and thickness in a more reliable way. Our study has some limitations. This is a heterogeneous group of patients and the presence of radiological OA could not be accounted for as radiographs were not performed. It would be useful to explore among larger cohort studies whether the influence of adipokines on articular cartilage varies as different stages of the OA process. We did not assess body composition, which is important as BMI does not discriminate adipose from non-adipose body mass, and evidence suggests fat and muscle contribute opposite effects to the pathogenesis of OA[47]. A further limitation was the potential impact of confounding medical conditions, such as insulin resistance state and dyslipidemia, or medications used, which was not assessed for in the current study. The association of leptin-to-adiponectin ratio, a possible surrogate marker of insulin resistance[48], with cartilage outcomes was not statistically significant. Blood and urine for adipokine and biomarker analysis were not taken in the fasted state; this has been shown to affect markers of bone resorption[49]. We did not standardize time of day collection; instead, subjects were assessed at the time of their MRI, given accessibility. Markers of cartilage turnover exhibit diurnal variation, although the postulated mechanism is physical activity – the biomarkers accumulate in the synovium of osteoarthritic joints during rest and are transported *via* the lymphatics to the circulation during activity[50]. As the original study also assessed delayed gadolinium-enhanced MRI of cartilage (dGEMRIC), all of our subjects were required to ambulate *prior to* MRI making this less likely to be a factor.

Overall, our study has demonstrated that weight loss is associated with increases in adiponectin and decreases in leptin; these changes in turn were associated with both reduced knee cartilage volume and thickness loss, These relationships were not independent of weight loss itself, suggesting that mechanical loading is the driving etiology of cartilage changes. Nonetheless, this suggests a possible additional mechanism for reduced cartilage loss in obese people who lose weight, as identified in our recent study[32]. The association between COMP and cartilage volume and thickness loss indicates it may be a useful joint biomarker.

Determining the metabolic mechanisms of obesity-related joint changes offers another potential approach towards the goal of disease-modifying therapy in OA, through the development of therapeutic strategies to counteract dysregulation of pro-inflammatory adipokine production and downstream events. Further work is required to determine the relative contributions of metabolic and mechanical factors in the pathogenesis of knee OA, and to explore additional parameters of metabolic change including insulin resistance.

Author contributions

LK, AA and LM were involved in the conception and design of the study. LK, HH, MS and AA were involved in the acquisition of data. LK, AA and LM were involved in the analysis and interpretation of data. All authors were involved in the drafting and revising of the article and have provided approval of the final version. LM and AA take responsibility for the integrity of the work as a whole, from inception to finished article.

Role of the funding source

We recognize the support of Arthritis Australia (assay analysis), NHMRC (MRI data, AA) and the Institute of Bone and Joint Research (MRI funding). These organizations had no involvement in the study design, collection, analysis and interpretation of data; in the writing of the manuscript; and in the decision to submit the manuscript for publication.

Conflict of interest

None of the authors have competing interests to disclose.

Acknowledgments

We acknowledge the late Philip N. Sambrook, MD, LLB, FRACP, for his critical role in the conception and design of this study. We thank Peter Forrest, Laboratory Manager, Diagnostic Pathology Unit at Concord Hospital for his assistance in biochemical analysis and the late Jian Sheng Charles Chen, PhD, for his statistical assistance.

References

1. Brooks PM. Impact of osteoarthritis on individuals and society: how much disability? Social consequences and health

economic implications. Curr Opin Rheumatol 2002 Sep;14(5):573–7.
2. Doherty M. Risk factors for progression of knee osteoarthritis. Lancet 2001 Sep 8;358(9284):775–6.
3. Anandacoomarasamy A, Caterson I, Sambrook P, Fransen M, March L. The impact of obesity on the musculoskeletal system. Int J Obes (Lond) 2008 Feb;32(2):211–22.
4. Spector TD, Hart DJ, Doyle DV. Incidence and progression of osteoarthritis in women with unilateral knee disease in the general population: the effect of obesity. Ann Rheum Dis 1994 Sep;53(9):565–8.
5. Szoeke C, Dennerstein L, Guthrie J, Clark M, Cicuttini F. The relationship between prospectively assessed body weight and physical activity and prevalence of radiological knee osteoarthritis in postmenopausal women. J Rheumatol 2006 Sep;33(9):1835–40.
6. Anandacoomarasamy A, Fransen M, March L. Obesity and the musculoskeletal system. Curr Opin Rheumatol 2009 Jan;21(1):71–7.
7. Oliveria SA, Felson DT, Cirillo PA, Reed JI, Walker AM. Body weight, body mass index, and incident symptomatic osteoarthritis of the hand, hip, and knee. Epidemiology 1999 Mar;10(2):161–6.
8. Gomez R, Conde J, Scotece M, Gomez-Reino JJ, Lago F, Gualillo O. What's new in our understanding of the role of adipokines in rheumatic diseases? Nat Rev Rheumatol 2011 Sep;7(9):528–36.
9. Garnero P, Rousseau JC, Delmas PD. Molecular basis and clinical use of biochemical markers of bone, cartilage, and synovium in joint diseases. Arthritis Rheum 2000 May;43(5):953–68.
10. Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. Nat Rev Immunol 2011 Feb;11(2):85–97.
11. Tilg H, Moschen AR. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. Nat Rev Immunol 2006 Oct;6(10):772–83.
12. Simopoulou T, Malizos KN, Iliopoulos D, Stefanou N, Papatheodorou L, Ioannou M, *et al*. Differential expression of leptin and leptin's receptor isoform (Ob-Rb) mRNA between advanced and minimally affected osteoarthritic cartilage; effect on cartilage metabolism. Osteoarthritis Cartilage 2007 Aug;15(8):872–83.
13. Figenschau Y, Knutsen G, Shahazeydi S, Johansen O, Sveinbjornsson B. Human articular chondrocytes express functional leptin receptors. Biochem Biophys Res Commun 2001 Sep 14;287(1):190–7.
14. Karsenty G. Convergence between bone and energy homeostases: leptin regulation of bone mass. Cell Metab 2006 Nov;4(5):341–8.
15. Bao JP, Chen WP, Feng J, Hu PF, Shi ZL, Wu LD. Leptin plays a catabolic role on articular cartilage. Mol Biol Rep 2010 Oct;37(7):3265–72.
16. Otero M, Gomez Reino JJ, Gualillo O. Synergistic induction of nitric oxide synthase type II: in vitro effect of leptin and interferon-gamma in human chondrocytes and ATDC5 chondrogenic cells. Arthritis Rheum 2003 Feb;48(2):404–9.
17. Otero M, Lago R, Lago F, Reino JJ, Gualillo O. Signalling pathway involved in nitric oxide synthase type II activation in chondrocytes: synergistic effect of leptin with interleukin-1. Arthritis Res Ther 2005;7(3):R581–91.
18. Vuolteenaho K, Koskinen A, Kukkonen M, Nieminen R, Paivarinta U, Moilanen T, *et al*. Leptin enhances synthesis of proinflammatory mediators in human osteoarthritic cartilage–mediator role of NO in leptin-induced PGE2, IL-6, and IL-8 production. Mediat Inflamm 2009;2009:345838.
19. Pallu S, Francin PJ, Guillaume C, Gegout-Pottie P, Netter P, Mainard D, *et al*. Obesity affects the chondrocyte responsiveness to leptin in patients with osteoarthritis. Arthritis Res Ther 2010;12(3):R112.
20. Gross JB, Guillaume C, Gegout-Pottie P, Mainard D, Presle N. Synovial fluid levels of adipokines in osteoarthritis: association with local factors of inflammation and cartilage maintenance. Biomed Mater Eng 2014;24(1 Suppl):17–25.
21. Koskinen A, Juslin S, Nieminen R, Moilanen T, Vuolteenaho K, Moilanen E. Adiponectin associates with markers of cartilage degradation in osteoarthritis and induces production of proinflammatory and catabolic factors through mitogen-activated protein kinase pathways. Arthritis Res Ther 2011;13(6):R184.
22. de Boer TN, van Spil WE, Huisman AM, Polak AA, Bijlsma JW, Lafeber FP, *et al*. Serum adipokines in osteoarthritis; comparison with controls and relationship with local parameters of synovial inflammation and cartilage damage. Osteoarthritis Cartilage 2012 May 14;20(8):846–53.
23. Richette P, Poitou C, Garnero P, Vicaut E, Bouillot JL, Lacorte JM, *et al*. Benefits of massive weight loss on symptoms, systemic inflammation and cartilage turnover in obese patients with knee osteoarthritis. Ann Rheum Dis 2011 Jan;70(1):139–44.
24. Rousseau JC, Delmas PD. Biological markers in osteoarthritis. Nat Clin Pract Rheumatol 2007 Jun;3(6):346–56.
25. van Spil WE, DeGroot J, Lems WF, Oostveen JC, Lafeber FP. Serum and urinary biochemical markers for knee and hip-osteoarthritis: a systematic review applying the consensus BIPED criteria. Osteoarthritis Cartilage 2010 May;18(5):605–12.
26. King KB, Lindsey CT, Dunn TC, Ries MD, Steinbach LS, Majumdar S. A study of the relationship between molecular biomarkers of joint degeneration and the magnetic resonance-measured characteristics of cartilage in 16 symptomatic knees. Magn Reson Imaging 2004 Oct;22(8):1117–23.
27. Ding C, Parameswaran V, Cicuttini F, Burgess J, Zhai G, Quinn S, *et al*. Association between leptin, body composition, sex and knee cartilage morphology in older adults: the Tasmanian older adult cohort (TASOAC) study. Ann Rheum Dis 2008 Sep;67(9):1256–61.
28. Bruyere O, Collette J, Kothari M, Zaim S, White D, Genant H, *et al*. Osteoarthritis, magnetic resonance imaging, and biochemical markers: a one year prospective study. Ann Rheum Dis 2006 Aug;65(8):1050–4.
29. Berry PA, Maciewicz RA, Wluka AE, Downey-Jones MD, Forbes A, Hellawell CJ, *et al*. Relationship of serum markers of cartilage metabolism to imaging and clinical outcome measures of knee joint structure. Ann Rheum Dis 2010 Oct;69(10):1816–22.
30. Lohmander LS, Brandt KD, Mazzuca SA, Katz BP, Larsson S, Struglics A, *et al*. Use of the plasma stromelysin (matrix metalloproteinase 3) concentration to predict joint space narrowing in knee osteoarthritis. Arthritis Rheum 2005 Oct;52(10):3160–7.
31. Pelletier JP, Raynauld JP, Caron J, Mineau F, Abram F, Dorais M, *et al*. Decrease in serum level of matrix metalloproteinases is predictive of the disease-modifying effect of osteoarthritis drugs assessed by quantitative MRI in patients with knee osteoarthritis. Ann Rheum Dis 2010 Jun 22;69(12):2095–101.
32. Anandacoomarasamy A, Leibman S, Smith G, Caterson I, Giuffre B, Fransen M, *et al*. Weight loss in obese people has

structure-modifying effects on medial but not on lateral knee articular cartilage. Ann Rheum Dis 2012 Jan;71(1):26–32.
33. Chua Jr SD, Messier SP, Legault C, Lenz ME, Thonar EJ, Loeser RF. Effect of an exercise and dietary intervention on serum biomarkers in overweight and obese adults with osteoarthritis of the knee. Osteoarthritis Cartilage 2008 Sep;16(9):1047–53.
34. Anandacoomarasamy A, Giuffre BM, Leibman S, Caterson ID, Smith GS, Fransen M, *et al.* Delayed gadolinium-enhanced magnetic resonance imaging of cartilage: clinical associations in obese adults. J Rheumatol 2009 May;36(5):1056–62.
35. Altman R, Asch E, Bloch D, Bole G, Borenstein D, Brandt K, *et al.* Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and therapeutic criteria committee of the American Rheumatism Association. Arthritis Rheum 1986 Aug;29(8):1039–49.
36. Eckstein F, Wirth W, Hudelmaier MI, Maschek S, Hitzl W, Wyman BT, *et al.* Relationship of compartment-specific structural knee status at baseline with change in cartilage morphology: a prospective observational study using data from the osteoarthritis initiative. Arthritis Res Ther 2009;11(3):R90.
37. Erhart-Hledik JC, Favre J, Asay JL, Smith RL, Giori NJ, Mundermann A, *et al.* A relationship between mechanically-induced changes in serum cartilage oligomeric matrix protein (COMP) and changes in cartilage thickness after 5 years. Osteoarthritis Cartilage 2012 Nov;20(11):1309–15.
38. Elsaid KA, Chichester CO. Review: collagen markers in early arthritic diseases. Clin Chim Acta 2006 Mar;365(1–2):68–77.
39. Miller GD, Nicklas BJ, Loeser RF. Inflammatory biomarkers and physical function in older, obese adults with knee pain and self-reported osteoarthritis after intensive weight-loss therapy. J Am Geriatr Soc 2008 Apr;56(4):644–51.
40. Fenske WK, Dubb S, Bueter M, Seyfried F, Patel K, Tam FW, *et al.* Effect of bariatric surgery-induced weight loss on renal and systemic inflammation and blood pressure: a 12-month prospective study. Surg Obes Relat Dis 2012 Apr 10.
41. Nicklas BJ, Ambrosius W, Messier SP, Miller GD, Penninx BW, Loeser RF, *et al.* Diet-induced weight loss, exercise, and chronic inflammation in older, obese adults: a randomized controlled clinical trial. Am J Clin Nutr 2004 Apr;79(4):544–51.
42. Stannus O, Jones G, Cicuttini F, Parameswaran V, Quinn S, Burgess J, *et al.* Circulating levels of IL-6 and TNF-alpha are associated with knee radiographic osteoarthritis and knee cartilage loss in older adults. Osteoarthritis Cartilage 2010 Nov;18(11):1441–7.
43. Livshits G, Zhai G, Hart DJ, Kato BS, Wang H, Williams FM, *et al.* Interleukin-6 is a significant predictor of radiographic knee osteoarthritis: the Chingford study. Arthritis Rheum 2009 Jul;60(7):2037–45.
44. Distel E, Cadoudal T, Durant S, Poignard A, Chevalier X, Benelli C. The infrapatellar fat pad in knee osteoarthritis: an important source of interleukin-6 and its soluble receptor. Arthritis Rheum 2009 Nov;60(11):3374–7.
45. Hunter DJ, Beavers D, Eckstein F, Guermazi A, Loeser RF, Nicklas BJ, *et al.* The Intensive Diet and Exercise for Arthritis Trial (IDEA): 18-Month Radiographic and MRI Outcomes. American College of Rheumatology Annual Meeting. Washington D.C., USA 2012.
46. Conaghan PG, Felson D, Gold G, Lohmander S, Totterman S, Altman R. MRI and non-cartilaginous structures in knee osteoarthritis. Osteoarthritis Cartilage 2006;14(Suppl A):A87–94.
47. Berry PA, Wluka AE, Davies-Tuck ML, Wang Y, Strauss BJ, Dixon JB, *et al.* The relationship between body composition and structural changes at the knee. Rheumatology (Oxford) 2010 Dec;49(12):2362–9.
48. Finucane FM, Luan J, Wareham NJ, Sharp SJ, O'Rahilly S, Balkau B, *et al.* Correlation of the leptin:adiponectin ratio with measures of insulin resistance in non-diabetic individuals. Diabetologia 2009 Nov;52(11):2345–9.
49. Christgau S. Circadian variation in serum CrossLaps concentration is reduced in fasting individuals. Clin Chem 2000 Mar;46(3):431.
50. Kong SY, Stabler TV, Criscione LG, Elliott AL, Jordan JM, Kraus VB. Diurnal variation of serum and urine biomarkers in patients with radiographic knee osteoarthritis. Arthritis Rheum 2006 Aug;54(8):2496–504.