Research Article

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Biomarkers in Medicine

FABP4 as a biomarker for knee osteoarthritis

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Aim: To explore the role of an adipokine-termed fatty acid-binding protein 4 (FABP4) in osteoarthritis (OA). **Methods:** Patients with primary knee OA and non-OA controls were included. Paired tissues including plasma, synovial fluid (SF), subcutaneous fat and infrapatellar fat pad (IPFP) were harvested during surgery. FABP4 concentration was determined by ELISA. **Results:** Plasma FABP4 increased significantly with OA stage (n = 263). OA patients (n = 38) had significantly higher plasma and SF FABP4 than non-OA patients (n = 29). FABP4 level of IPFP was positively correlated with SF FABP4. **Conclusion:** OA patients had significantly high systemic and local FABP4, and IPFP may be the main source of FABP4 in synovial cavity. FABP4 may be a promising biomarker for OA.

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Keywords: FABP4 • infrapatellar fat pad • osteoarthritis

Osteoarthritis (OA) is a serious joint disorder that affects millions of people worldwide, especially the elderly individuals. It was the most common form of arthritis, and was estimated to be the fourth leading cause for disability by the year 2020 [1]. OA is characterized structurally by articular cartilage degradation and remodeling of the underlying subchondral bone, and is accompanied by inflammation, pain and functional loss of the joint [2]. Current treatments for OA are mainly symptomatic treatments with various drawbacks.

The etiology of OA is still poorly understood. Genetics, injury and obesity are well-recognized risk factors for OA. Obesity is one modifiable risk factor, and is conventionally considered to cause OA via the mechanical loading on the joint. However, the theory of loading (also known as 'wear and tear') cannot answer the question of why OA is also found in nonweight-bearing joints such as hand [3–5]. Increasing evidence has shown that obesity also contributes to cartilage degeneration by producing and releasing a plethora of factors termed adipokines [6]. Leptin, adiponectin, resistin and visfatin have been reported to induce expression of inflammatory cytokines in chondrocyte, such as nitric oxide synthase and prostaglandin E2 [7–10], which generated degradation of extracellular matrix.

FABP4, also known as adipocyte FABP (aP2), is an adipokine that is mainly expressed in adipocyte and macrophage [11]. The function of FABP4 is to act as lipid chaperon, which actively facilitates the transportation of lipids to specific compartments in the cell [11]. However, high concentration of FABP4 has been found to be closely associated with obesity and metabolic diseases, including Type 2 diabetes, atherosclerosis or coronary heart disease [12–16]. On the other hand, the use of a FABP4 selective oral inhibitor (BMS309403) in mice could significantly decrease the risk of arthrosclerosis [17].

Given above, it poses possibility that FABP4 may play roles in linking obesity to OA. However, to our knowledge, no studies have investigated the role of FABP4 in OA. And therefore, the purpose of this study was to discover the potential role of FABP4 in OA. In the first study, we included patients with different stage of primary knee OA, and determined their plasma FABP4 concentration. We aimed to explore the correlation of plasma FABP4 with the severity of knee OA. In the second parallel study, we included patients with end-stage knee OA who required total knee replacement surgery and non-OA patients with knee injury who required knee arthroscopic surgery, and harvested paired tissue samples including plasma, synovial fluid (SF), subcutaneous fat (ScAT) and infrapatellar fat pad (IPFP) during surgery. The purpose was to investigate if the expression pattern of FABP4 was significantly different between OA patients and non-OA controls.

Materials & methods

Participants & collection of blood

This study was approved by the institutional review board of the University of Hong Kong and Hospital Authority Hong Kong West Cluster (HKU/HA HKW IRB, no.: UW13–251). Informed consent was obtained by signing consent forms from all patients participated. All the participants are southern Chinese population. We performed two parallel studies. In the first study, patients came to our departmental outpatient clinic complaining of knee pain and whose knee x-rays had confirmed of primary knee OA were prospectively included. The severity of knee OA was evaluated according to the Kellgren & Lawrence (KL) knee OA grading system [18]. Briefly, knee OA was classified into five grades (0–4): Grade 0: no radiographic features of OA are present; Grade 1: doubtful joint space narrowing (JSN) and possible osteophytic lipping; Grade 2: definite osteophytes and possible JSN on anteroposterior weight-bearing radiograph; Grade 3: multiple osteophytes, definite JSN, sclerosis and possible bony deformity. Grade 4: large osteophytes, marked JSN, severe sclerosis and definite bony deformity. Patients with KL-0 were considered as control to OA patients. Only patients with primary knee OA, either unilateral or bilateral were included. In case of inconsistent KL grades between left and right knee, the higher grade was selected. Patients with traumatic OA, rheumatoid arthritis (RA), septic arthritis or patients who were on medication for hypertension, diabetes and lipidemia were excluded. All patients' demographic information, including gender, age, body height, body weight and BMI was recorded. Patients were classified as nonobese (BMI <25), overweight (BMI $25 <30$) and obese (BMI ≥ 30) patients according to the WHO definition. Fasting vein blood were taken in EDTA tubes, and transferred to laboratory immediately. Blood were centrifuged at 2500 rpm in 4°C for 10 min. The supertanant (plasma) were harvested and stored in aliquots at -80°C until further analysis.

In the second study, patients with advanced knee OA (KL-3 and KL-4) who were admitted to our department for total knee replacement surgery, and patients who had knee injury (anterior cruciate ligament tear, collateral ligament tear or meniscus tear) who required knee arthroscopic surgery but x-rays showed no primary knee OA signs were prospectively included (as control). For the OA patients, only primary OA were included. For the non-OA controls, only patients whose injures happened 3 months before surgery were included to rule out the confounding factors of acute inflammation. Patients with traumatic OA, RA, septic arthritis or patients who were on medication for hypertension, diabetes and lipidemia were excluded. Fasting vein blood was taken 1 day before surgery, and plasma were harvested same as described in the first study.

Collection of SF & adipose tissues

During surgery, prior to skin incision, joint aspiration was performed and 1 ml of SF was taken. After skin incision, paired adipose tissues including ScAT and IPFP were dissected. Samples were transferred immediately to laboratory in icebox. SF was centrifuged at 14,000 rpm in 4°C for 15 min to discard cell debris. The supernatant were collected and stored in aliquots at -80°C until further analysis. The adipose tissues were washed three-times with sterile saline and processed for tissue explant culture. Briefly, tissues were dissected into three pieces of approximately 10 mg, and immediately seeded into 12-well cell culture plate with 2.0 ml of Dulbecco's Modified Eagle Cell Culture Medium containing 10% bull serum albumin (fatty acid free; A8806, Sigma, MO, USA), and incubated at 37°C with 5% CO_2 for 24 h. Afterward, the medium was harvested and centrifuged at 14,000 rpm in 4°C for 10 min. The supernatant was harvested and stored in aliquots at -80°C until further analysis.

Determination of FABP4

In the first study, plasma FABP4 was determined with an ELISA commercial kit (DFBP40, R&D Systems, MN, USA) according to the manual book provided. Intra-assay and interassay coefficients of variation were 4.6 and

Table 1. FABP4 level of 263 participants diagnosed with different stage of primary knee osteoarthritis.

Characteristic	Non-OA	OA			p-value
	KL grade 0	KL grade 2	KL grade 3	KL grade 4	
n	15 (3M, 12F)	72 (20M, 52F)	65 (12M, 53F)	111 (29M, 82F)	–
Age (years)	59.6 ± 5.5 (56.6–62.6)	57.6 ± 8.4 (55.6–59.6)	64.1 ± 7.9 (62.1–66.0)	67.5 ± 8.6 (65.8–69.2)	1.4×10^{-5}**
BMI	25.4 ± 3.5 (23.4–27.5)	25.2 ± 4.1 (24.2–26.2)	27.0 ± 4.6 (25.9–28.2)	27.2 ± 4.3 (26.3–28.1)	0.013*
Plasma FABP4 (ng/ml)	12.3 ± 3.1 (10.6–14.0)	14.6 ± 6.9 (13.0–16.3)	18.2 ± 11.3 (15.4–21.0)	19.4 ± 12.0 (17.0–21.7)	0.005*

Data were presented as mean ± standard deviation with 95% CI.
*$p < 0.05$.
**$p > 1$.
BMI: Body mass index; F: Female; KL: Kellgren and Lawrence; M: Male; OA: Osteoarthritis.

12.1%, respectively. Plasma underwent 20-fold dilution before analysis. In the second study, the FABP4 concentration of plasma, SF and culture medium of ScAT and IPFP was determined with another FABP4 quantitative ELISA kit made by our own institution (31030, Antibody and Immunoassay Services, HKU). Intra-assay and interassay coefficients of variation were less than 4.1 and 4.5%, respectively. Specifically, SF after thawed was pretreated with 2 mg/ml hyaluronidase (H3506, Sigma) at room temperature for 1 h on a shaker. Plasma, SF and culture medium of ScAT and IPFP underwent threefold dilution, tenfold dilution and 400-fold dilution before analysis, respectively. The FABP4 concentration from adipose tissues was adjusted by the weight of seeded tissue, and the mean value of three wells from same tissue was calculated. The coefficient of variation (CV) was calculated, defined as the ratio of standard deviation (SD) divided by mean of the values of three wells. If CV >15%, data were considered as largely variable and therefore were not included in the statistical analysis.

Statistical analyses

Data were presented as mean ± SD with 95% CI. Data normality was determined by the Shapiro–Wilk test. Any data that were not normally distributed were logarithmically transformed prior to analysis. One-way analysis of variance (ANOVA) was performed to compare the difference of plasma FABP4 concentration among different BMI categories and KL groups, followed by ordinal logistic regression analysis aiming to adjust the confounding factors of age, sex and BMI. Receiver operating characteristic (ROC) curve was calculated to examine area under the curve and establish optimal cutoff value of FABP4 for knee OA. Student's t-test was used to compare the difference of FABP4 concentration of plasma, SF and culture medium of adipose tissues between OA patients and non-OA controls, followed by univariate linear regression analysis that aimed to adjust the confounding factors of age, sex and BMI. Paired t-test was used to compare the difference between SF and plasma FABP4 concentration in OA patients or non-OA controls. Bivariate correlation analysis was performed to examine the correlation of plasma FABP4, SF FABP4 and paired adipose tissues FABP4 with BMI after adjusted by age and sex. Two-sided p-value of less than 0.05 was considered statistically significant. Analyses were performed in statistical software SPSS 23.0 (IBM Corporation, NY, USA).

Results

Plasma FABP4 was closely associated with BMI & the severity of knee OA

In the first study, a total of 263 patients were included. There were 64 males and 199 females, with a mean age of 63.3 ± 9.2 years (95% CI: 62.2–64.5) and a mean BMI of 26.5 ± 4.4 (95% CI: 25.9–27.0). Fifteen patients were KL-0 (non-OA), 72 patients were KL-2, 65 patients were KL-3 and 111 patients were KL-4. The demorgraphics of patients were summarized in Table 1.

According to the WHO definition, 99 patients were nonobese, 96 patients were overweight and 68 patients were obese patients. The mean FABP4 concentration of these patients was 14.4 ± 8.3, 17.9 ± 9.5 and 21.1 ± 13.5 ng/ml, respectively ($p = 1.1 \times 10^{-4}$, one-way ANOVA). Post-hoc analysis showed the obese patients had significantly higher plasma FABP4 than the nonobese patients ($p = 1.0 \times 10^{-6}$, Tukey HSD; Figure 1A). The partial correlation analysis showed plasma FABP4 was positively correlated with BMI after adjusting age and sex (Pearson's coefficient: 0.314; $p < 0.001$).

The mean plasma FABP4 concentration of KL-0, KL-2, KL-3 and KL-4 patients was 12.3 ± 3.1, 14.6 ± 6.9, 18.2 ± 11.3 and 19.4 ± 12.0 ng/ml, respectively ($p = 0.005$, one-way ANOVA). The post-hoc analysis showed the concentration of KL-4 patients was significantly higher than the patients of KL-2 ($p = 0.02$, Turkey HSD) and

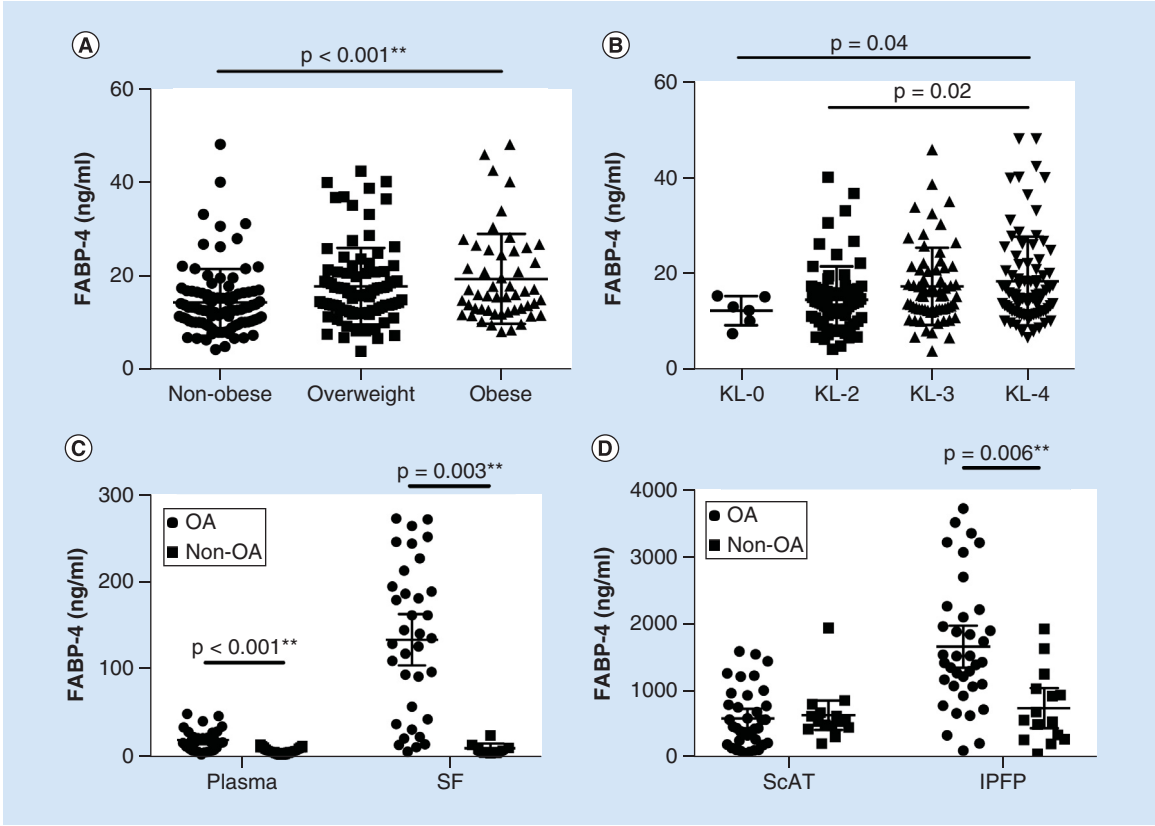

Figure 1. FABP4 concentration of paired tissue samples from osteoarthritis patients and nonosteoarthritis controls.
(A) Plasma FABP4 concentration of 263 patients. The obese patients had significantly higher plasma FABP4 concentration than nonobese patients ($p < 0.001$). **(B)** Plasma FABP4 concentration of 263 patients with different KL grades. FABP4 concentration was significantly different among different KL groups ($p = 0.005$, one-way ANOVA). The post-hoc analysis showed the concentration of KL-4 patients was significantly higher than the patients of KL-2 ($p = 0.02$) and KL-0 ($p = 0.04$). **(C)** Plasma and synovial fluid FABP4 concentration of 30 OA patients (KL-3 and KL-4) and 25 non-OA patients (KL-0). The OA patients had significantly higher concentration of plasma FABP4 ($p < 0.001$) and synovial FABP4 ($p = 0.003$) compared with the non-OA patients. **(D)** ScAT and IPFP expression concentration of FABP4 in 38 OA patients and 15 non-OA patients. The FABP4 expression level of ScAT was not significantly different between OA and non-OA patients. However, the level of IPFP was significantly higher in OA patients ($p = 0.006$). Data were presented as mean with 95% CI (*$p < 0.05$, **$p < 0.01$).
ANOVA: Analysis of variance; IPFP: Infrapatellar fat pad; KL: Kellgren and Lawrence; OA: Osteoarthritis; ScAT: Subcutaneous fat.

KL-0 ($p = 0.04$, Turkey HSD; Figure 1B). In order to further investigate the correlation of FABP4 concentration and the severity of knee OA, we stratified FABP4 concentration into four groups (<10, 10–15, 15–20, >20 ng/ml) and performed the ordinal logistic regression analysis. Results showed patients with plasma FABP4 of less than 10 ng/ml had an estimated probability of 3.53 of diagnosing with a lower KL grade of OA than patients with FABP4 of more than 20 ng/ml ($p = 0.03$, age and sex adjusted). And patients with plasma FABP4 between 15 and 20 ng/ml had an estimated probability of 2.10 of diagnosing with a lower KL grade of OA than patients with FABP4 of more than 20 ng/ml ($p = 0.04$, age and sex adjusted).

Sensitivity and specificity of different cutoff value of FABP4 was calculated, and the ROC curve was drawn (Figure 2). The area under the curve was 0.68. The optimum cutoff value for FABP4 is 15.45 ng/ml, with 48% of sensitivity and 93% of specificity achieving maximum Youden index of 0.41.

FABP4 concentration of plasma, SF & IPFP was significantly higher in OA patients than non-OA patients

In the second study, a total of 67 patients were recruited, including 38 OA patients (KL-3 and KL-4) and 29 non-OA patients (KL-0). There were 37 males and 30 females, with a mean age was 52.6 ± 22.9 years (95% CI:

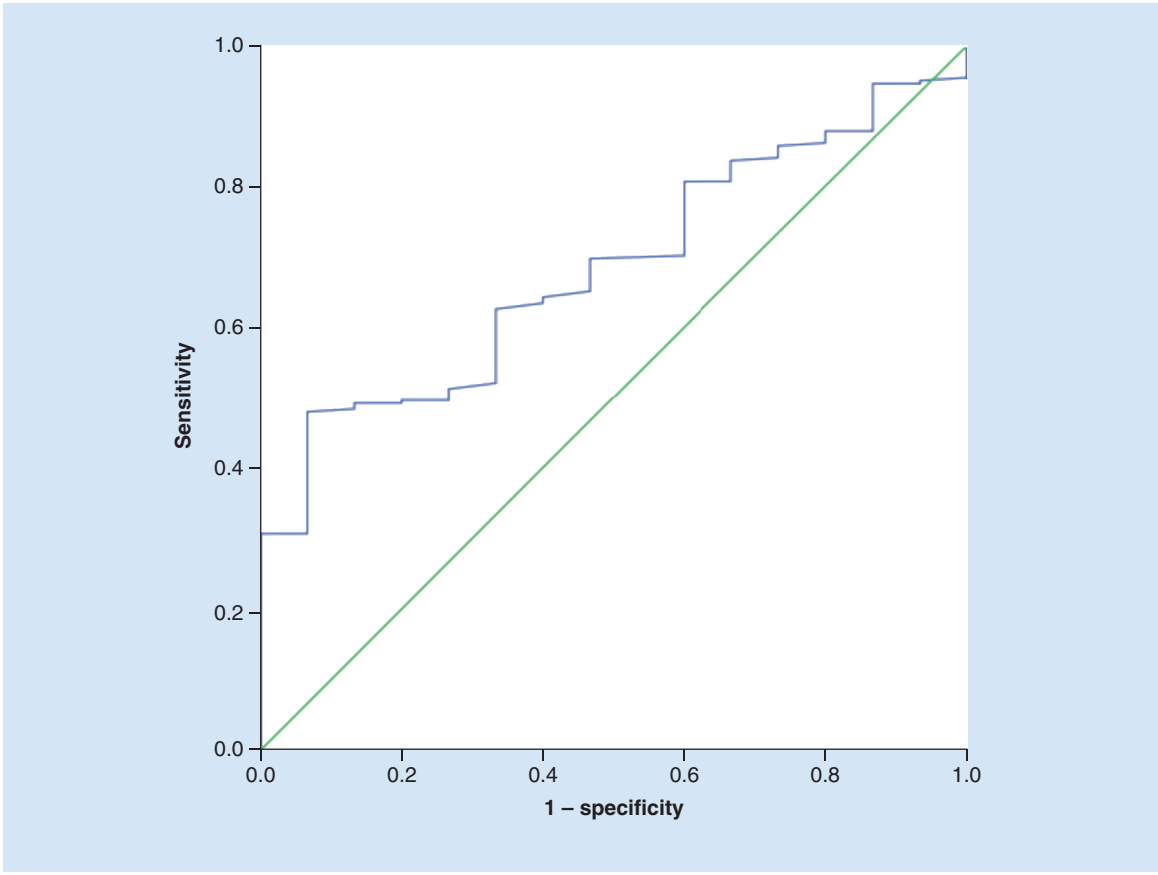

Figure 2. A receiver operating characteristic curve for plasma fatty acid-binding protein 4 for diagnosing primary knee osteoarthritis is shown with an area under the curve of 68%. A cut off value of 15.45 ng/ml demonstrates 48% sensitivity and 93% specificity. Diagonal segments are produced by ties.

47.0–58.2) and average BMI of 27.5 ± 4.6 (95% CI: 26.2–28.7). The demorgraphics of patients were summarized in Table 2.

The OA patients had significantly higher concentration of plasma FABP4 compared with the non-OA patients (18.3 ± 12.4 vs 5.4 ± 3.2 ng/ml, $p = 6.2 \times 10^{-6}$; Figure 1C). The univariate general linear analysis showed after adjusting sex and BMI, FABP4 was still significantly higher in OA group than non-OA group (adjusted estimated concentration: OA 16.7 ± 1.9 ng/ml vs non-OA 7.8 ± 2.2 ng/ml, $p = 0.001$). However, there was no significant difference after adjusting sex, BMI and age simultaneously ($p = 0.34$).

The FABP4 of SF in OA patients was also significantly higher than the non-OA controls (133.2 ± 85.8 vs 8.5 ± 6.8 ng/ml, $p = 0.003$; Figure 1C). Again, the univariate general linear analysis showed significantly higher FABP4 in OA group than non-OA group after adjusting sex and BMI (estimated concentration: OA 125.8 ± 17.2 ng/ml vs non-OA 40.7 ± 55.2 ng/ml, $p = 0.01$), but significance was lost after adjusting sex, BMI and age simultaneously ($p = 0.36$).

For paired adipose tissues, the mean CV of tissue replicates was 11.3 ± 2.7%. The FABP4 expression level of ScAT was not significantly different between OA patients and non-OA controls (OA: 746.2 ± 910.7 ng/ml vs non-OA 643.5 ± 394.0 ng/ml, $p = 0.68$; Figure 1C). However, the FABP4 expression level of IPFP was significantly higher in OA patients than non-OA controls (1857.5 ± 1331.6 vs 749.7 ± 537.0 ng/ml, $p = 0.006$; Figure 1D). Consistent to plasma and SF, the concentration was still significantly different after adjusting sex and BMI (estimated concentration: OA 1709.6 ± 306.9 ng/ml vs non-OA 1124.4 ± 680.4 ng/ml, $p = 0.03$) but not different after adjusting sex, BMI and age simultaneously ($p = 0.87$).

In OA patients, the synovial FABP4 was significantly higher than paired plasma FABP4 (133.2 ± 85.8 vs 18.3 ± 12.4 ng/ml, paired t test; $p = 0.001$), while not significantly different in non-OA patients (SF: 8.5 ± 6.8 ng/ml vs plasma: 5.4 ± 3.2 ng/ml; $p = 0.41$; Figure 3A & B). The FABP4 expression concentration of

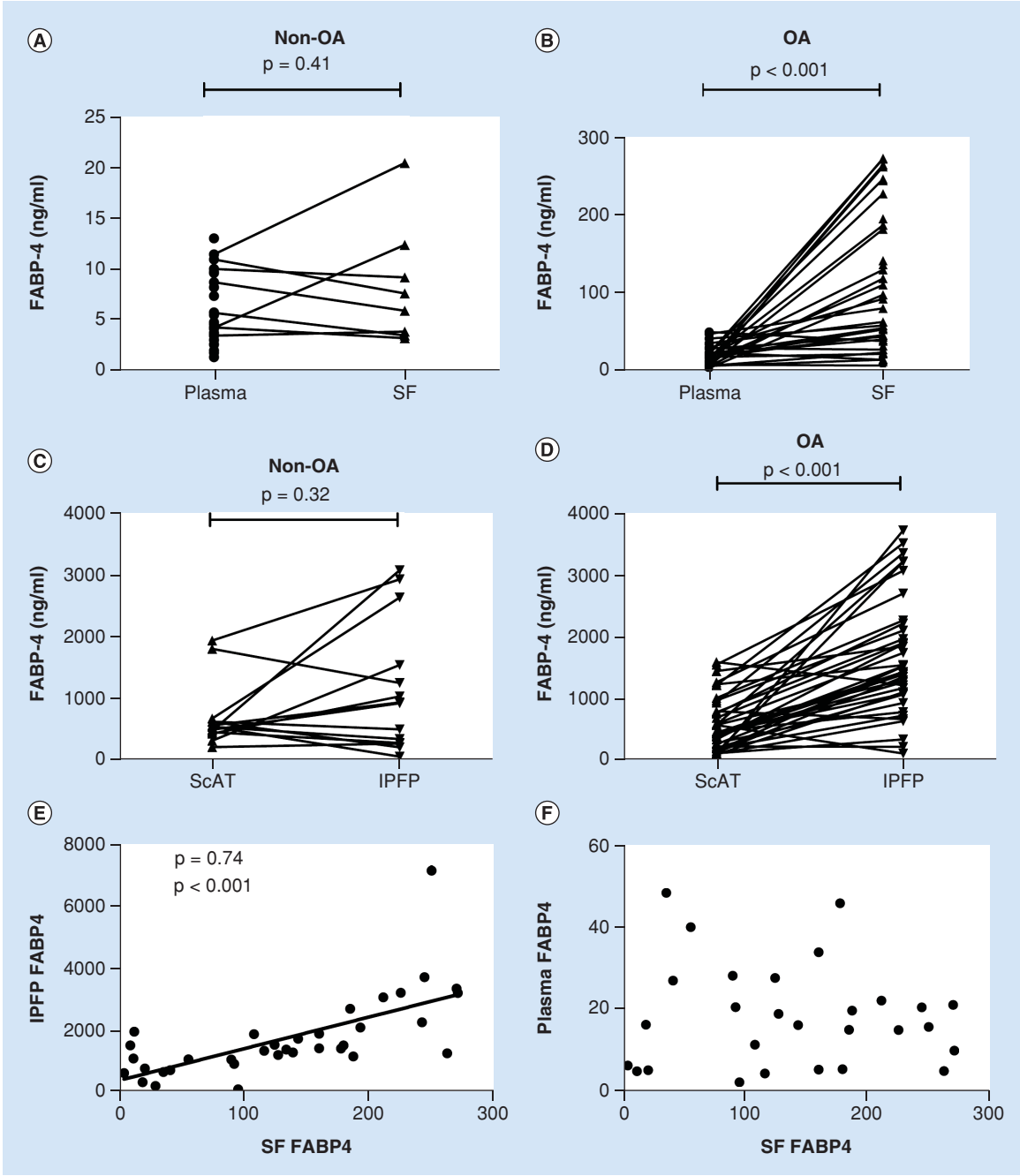

Figure 3. FABP4 expression level of paired tissue samples including plasma, synovial fluid, infrapatellar fat pad and subcutaneous fat. (A) In non-OA patients, synovial FABP4 was not significantly different than plasma FABP4 ($p = 0.41$). **(B)** In OA patients, the synovial FABP4 was significantly higher than paired plasma FABP4 ($p < 0.001$). **(C)** In non-OA patients, FABP4 expression level of IPFP was not significantly different than the level of ScAT ($p = 0.32$). **(D)** However, in OA patients, the FABP4 expression level of IPFP was significantly higher than the expression level of ScAT ($p < 0.001$). **(E)** Partial correlation analysis showed SF FABP4 was positively correlated with IPFP FABP4 in OA patients after adjusting age, sex and BMI (Pearson coefficient: 0.74, $p < 0.001$). **(F)** However, SF FABP4 had no correlation with plasma FABP4 in OA patients.
IPFP: Infrapatellar fat pad; OA: Osteoarthritis; ScAT: Subcutaneous fat; SF: Synovial fluid.

Table 2. FABP4 level of paired tissue samples including plasma, synovial fluid, subcutaneous fat and infrapatellar fat pad from 29 nonosteoarthritis patients and 38 osteoarthritis patients.

Patients	Non-OA	OA	p-value
n	29 (25 M, 4 F)	38 (12 M, 26 F)	–
Age	28.3 ± 7.3 (24.8–31.3)	71.2 ± 9.0 (65.1–73.5)	1.0×10^{-7}**
BMI	25.4 ± 3.3 (24.0–26.9)	28.9 ± 4.9 (27.4–31.2)	0.004**
Plasma			
– n	25	30	6.2×10^{-6}**
– FABP4 (ng/ml)	5.4 ± 3.2 (1.8–11.7)	18.3 ± 12.4 (13.7–22.9)	
SF:			
– n	8	35	2.0×10^{-4}**
– FABP4 (ng/ml)	8.5 ± 6.8 (-0.4 to 20.3)	133.2 ± 85.8 (106.3–171.9)	
ScAT:			
– n	15	38	0.68
– FABP4 (ng/ml)	643.5 ± 394.0 (177.9–790.3)	746.2 ± 910.7 (330.1–1138.2)	
IPFP:			
– n	15	38	0.003**
– FABP4 (ng/ml)	749.7 ± 537.0 (70.2–679.2)	1857.5 ± 1331.6 (1292.3–2440.9)	

Data were presented as mean ± standard deviation with 95% CI.
F: Female; FABP4: Fatty acid-binding protein 4; IPFP: Infrapatellar fat pad; M: Male; OA: Osteoarthritis; ScAT: Subcutaneous fat; SF: Synovial fluid.

IPFP was significantly higher than ScAT in OA patients (1857.5 ± 1331.6 vs 746.2 ± 910.7 ng/ml; $p = 0.008$), while no such difference was found in non-OA patients (ScAT 643.5 ± 394.0 vs IPFP 749.7 ± 537.0, $p = 0.32$; Figure 3C & D). Partial correlation analysis showed SF FABP4 was positively correlated with IPFP FABP4 in OA patients after adjusting age, sex and BMI (Pearson coefficient: 0.74; $p < 0.001$), but did not show a correlation between SF FABP4 and plasma FABP4 ($p = 0.65$; Figure 3E & F).

Discussion

OA is the most common form of arthritis. However, the etiology of OA is still poorly understood. Obesity is one of the modifiable risk factors for OA, and recent studies have suggested that adipokines play important roles in linking obesity to OA [19]. In this study, we aimed at exploring the potential role of a novel adipokine termed FABP4 in OA. We found that the plasma FABP4 was closely associated with BMI, and the concentration increased significantly with the severity of knee OA. OA patients had significantly higher systemic FABP4 (plasma) and local FABP4 (SF and IPFP) compared with non-OA patients, after adjusted for gender and BMI. In OA patients, the IPFP secretes significantly higher FABP4 than ScAT, and the concentration was positively correlated with the synovial FABP4. These data suggest that high concentration of FABP4 is associated with the severity of knee OA, and IPFP may be the main source of FABP4 in the synovial cavity.

FABP4 was first detected in adipose tissue and mature adipocytes in the 1980s [20], and is mainly expressed in adipocytes and macrophages [11]. Our study showed FABP4 was closely associated with BMI, which was in accordance with previous reports [12] and reaffirmed FABP4 as an adipokine. The function of FABP4 is to transport free fatty acid in adipocyte for different metabolic process [11]. However, recent studies have suggested high concentration of FABP4 as a risk factor for metabolic diseases in human [12–16]. Animal studies have shown that genetically knocking out FABP4 could protect mice from artherosclerosis, insulin resistance, diabetes and fatty liver diseases [21–24]. The detailed mechanism is not yet known but pilot studies demonstrated that FABP4 may be involved in the macrophage-mediated inflammatory responses [25].

However, if FABP4 plays roles in musculoskeletal diseases remains unknown. Cerezo *et al.* compared the FABP4 concentrations of serum and SF in RA patients and OA patients [26]. They found that the serum and SF FABP4 were significantly higher in RA patients than OA patients, which suggest FABP4 as a biomarker for RA. However, in their study, the OA patients were treated as control cohort to RA patients. We also noted the mean serum FABP4 level in their study was 19.6 ± 8.4 ng/ml, which was similar to our OA cohort (18.3 ± 12.4 ng/ml), however, the SF FABP4 in their study (18.04 ± 6.20 ng/ml) was significantly lower than ours (133.2 ± 85.8 ng/ml). The reason for this disparity may be due to the participants included. Our study included only the patients with advanced

stage of knee OA who required joint replacement surgery while Cezero *et al.*'s study, though not specified, probably included patients with early-stage of knee OA. It has been indicated in advanced OA joints, SF protein levels may be elevated by altered microvascular permeability due to inflammation, decreased consumption or lymphatic drainage [27].

The association of adipokines and the severity of knee OA have been investigated in previous studies. Staikos *et al.* looked into the correlation of plasma leptin with the severity of knee OA, and found a strong positive correlation [27]. De Boer *et al.*'s study also demonstrated that serum leptin and resistin concentrations were evidently increased in knee OA patients as compared with controls, independent of age and BMI [28]. Our previous study had shown that adiponectin was not significantly different between IPFP and ScAT media, but was higher in plasma than SF [29,30]. These data suggest adiponectin as an anti-inflammatory adipokine, while the current study provided new evidence that FABP4 may be a proimflammatory adipokine that is responsible for the development and progression of knee OA. However, although we have shown that plasma, SF and IPFP FABP4 concentration was significantly higher in OA patients than non-OA patients after adjusted for sex and BMI, the significance was lost after adjusting for sex, BMI and age simultaneously. This was probably due to the huge age gap between the OA cohort and non-OA cohort (71.2 ± 9.0 vs 28.3 ± 7.3). In this study, the OA patients were mostly elderly people with advanced OA while the non-OA patients were young or middle-aged patients who had sports-associated knee injuries. Nevertheless, this is the best control cohort we could find if we were to harvest paired tissue samples during surgery. On the other hand, the correlation between FABP4 concentration and age is still controversial. Bao *et al.*'s study reported that the circulating FABP4 concentration was positively correlated with age [31]. However, studies by Furuhashi *et al.* and Zhang *et al.* both showed that age was not significantly correlated with circulating FABP4 [32,33]. Further studies with larger sample size are required to elucidate the correlation between FABP4 and age.

The IPFP is a special adipose tissue that is located between the lower surface of the patella and the trochlear surface of the femur [34]. IPFP was initially thought to only have biomechanical function [35]. However, recent evidence suggests that IPFP is potential source of adipokines and cytokines at the articular level that presents a truly inflammatory phenotype in patients with OA [34,36–37]. IPFP has now been considered as an active periarticular tissue able to secrete several soluble factors that could contribute to the pathogenesis of OA [38,39]. Conde *et al.* have demonstrated recently that IPFPs from OA patients expressed higher levels of leptin and chemerin in comparison to healthy tissues [40]. Microarray analysis of IPFPs from end-stage OA patients have showed an increased expression of leptin and adiponectin as compared with early-stage OA patients tissues [41]. In addition to adipokines, IPFP was also reported to secrete interleukins and growth factors that could affect cartilage biology by promoting the expression of matrix metalloproteinases (MMPs) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) in chondrocyte [39]. Our study showed that FABP4 concentration was not significantly different in ScAT tissues between OA and non-OA controls, but FABP4 concentration of IPFP was significantly higher in OA patients than non-OA controls. In addition, FABP4 of IPFP was positively correlated with synovial FABP4. These data suggest that IPFP may be the main source of synovial FABP4. However, it was noted that the SF FABP4 in OA patients was 15-times higher than the non-OA patients, while IPFP FABP4 in OA patients was only two-times higher than the non-OA patients. This inconsistent ratio also indicates complicated inflammatory status in advanced OA joints. In end-stage OA joints, the IPFP is in severe inflammation phenotype that may affect its expression level of specific adipokines/cytokines. On the other hand, altered microvascular permeability due to inflammation may result in decreased consumption or drainage of SF proteins that may significantly increase their detectable levels [27].

High concentrations of synovial FABP4 may directly induce cartilage degeneration. Studies have shown that high concentrations of FABP4 could upregulate the expression of inflammatory cytokines including IL-1β, IL-6, MCP-1 and TNF-α [42], which were some of the critical mediators for the pathogenesis of OA. IL-1β and TNF-α, in particular, could induce the breakdown of cartilage matrix by stimulating the expression of MMPs and ADAMTS in chondrocyte [43,44]. Based on all of our study findings, we hypothesized that in OA patients, the IPFP had a changed phenotype that would synthesize and secrete high concentration of FABP4 into the synovial cavity. High synovial FABP4 could upregulate the expression levels of proinflammatory cytokines including IL-6, TNF-α, IL-1β or MCP-1 in chondrocyte, which further promotes the expression of MMPs and ADAMTS that degrade cartilage matrix. However, the detailed mechanism has to be tested in future studies. Our study also showed that patients with plasma FABP4 of less than 10 ng/ml had an estimated probability of 3.53 of diagnosing with a lower KL grade of OA than patients with FABP4 of more than 20 ng/ml. The probability, though not high, is an encouraging data as systemic use of chemicals or antibodies that target FABP4 may serve as a potential drug therapy for OA.

Furuhashi *et al.* had identified a FABP4 selective oral inhibitor (BMS309403) that could significantly decrease the risk of arthrosclerosis in mice [17]. If BMS304903 could be used to treat or prevent OA remains interesting to investigate.

Several limitations of this study have to be acknowledged. First, as mentioned patients with knee injury may not serve as the best control cohort to OA patients, as there exists age gap, and their periarticular tissues may not be considered as 'normal'. Second, this study was merely a cross-sectional observational study. It remains to be seen if high FABP4 could predict knee OA in longitudinal study. We also need to increase our sample size if to explore the potential of FABP4 as a diagnostic tool for primary knee OA.

Conclusion

We concluded from this study that primary knee OA patients had significantly higher systemic (plasma) and local (synovial) FABP4 than non-OA patients, and the systemic level increased significantly with the severity of knee OA. The IPFP is the main source of FABP4 in the synovial cavity. FABP4 may be a promising biomarker for the diagnosis, disease monitoring and treatment of knee OA.

Future perspective

OA is the most common form of arthritis but the etiology is still unknown. Current treatments for OA are mainly symptomatic treatments with various drawbacks. To discover a biomarker that can be used as diagnostic tool or as treatment target for OA is crucial. Our study provided novel insights that adipokine FABP4 may be a potential biomarker for OA. In the future, more studies have to be performed to investigate the detailed mechanism of how FABP4 induces the degeneration of cartilage. Transgenic mice (i.e., FABP4 knockout mice) can be utilized to explore the direct effects of FABP4 on the development and progression of OA. Furthermore, as inhibitors and antibodies of FABP4 are currently available in the market, if these drugs or chemicals can be used to treat OA remains interesting to investigate. These treatments will not simply be symptomatic treatments, but rather disease-modifying therapies.

Summary points

Background

- Adipokines are key mediators in the pathogenesis of osteoarthritis (OA).
- We aimed to discover the potential role of a novel adipokine termed FABP4 in OA.

Methods

- Patients with different stage of knee OA were prospectively included. Fasting blood were obtained. FABP4 concentration of plasma was determined.
- Patients with advanced knee OA required total knee replacement surgery and patients without knee OA but had knee injury that required arthroscopic surgery were prospectively enrolled. Paired tissue samples including plasma, synovial fluid (SF), subcutaneous fat and infrapatellar fat pad (IPFP) were harvested during surgery. Explant culture of adipose tissues were performed. FABP4 concentration of culture medium was determined.

Results

- Plasma FABP4 was positively correlated with BMI and increased significantly with the severity of knee OA.
- OA patients had significantly higher systemic FABP4 (plasma) and local FABP4 (SF and IPFP) than non-OA patients, after adjusted for gender and BMI. In OA patients, the expression level of IPFP FABP4 was positively correlated with the concentration of SF FABP4.

Conclusion

- FABP4 is an adipokine that may also play important roles in OA.
- FABP4 may be a promising biomarker for the diagnosis, disease monitoring or treatment target for knee OA.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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