Relationship Between Blood Plasma and Synovial Fluid Metabolite Concentrations in Patients with Osteoarthritis

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ABSTRACT. ***Objective.*** To investigate the relationship between plasma and synovial fluid (SF) metabolite concentrations in patients with osteoarthritis (OA).

Methods. Blood plasma and SF samples were collected from patients with primary knee OA undergoing total knee arthroplasty. Metabolic profiling was performed by electrospray ionization tandem mass spectrometry using the AbsoluteIDQ kit. The profiling yielded 168 metabolite concentrations. Correlation analysis between SF and plasma metabolite concentrations was done on absolute concentrations as well as metabolite concentration ratios using Spearman's rank correlation (ρ) method.

Results. A total of 69 patients with knee OA were included, 30 men and 39 women, with an average age of 66 ± 8 years. For the absolute metabolite concentrations, the average ρ was 0.23 ± 0.13. Only 8 out of 168 metabolite concentrations had a $\rho \geq 0.45$, with a p value $\leq 2.98 \times 10^{-4}$, statistically significant after correcting multiple testing with the Bonferroni method. For the metabolite ratios (n = 28,056), the average ρ was 0.29 ± 0.20. There were 4018 metabolite ratios with a $\rho \geq 0.52$ and a p value $\leq 1.78 \times 10^{-6}$, significant after correcting multiple testing. Sex-separate analyses found no difference in ρ between men and women. Similarly, there was no difference in ρ between people younger and older than 65 years.

Conclusion. Correlation between blood plasma and SF metabolite concentrations are modest. Metabolite ratios, which are considered proxies for enzymatic reaction rates and have higher correlations, should be considered when using blood plasma as a surrogate of SF in OA biomarker identification. (First Release March 1 2015; J Rheumatol 2015;42:859–65; doi:10.3899/jrheum.141252)

Key Indexing Terms:
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Metabolomics has become an ideal method for osteoarthritis (OA) biomarker identification[1]. A range of biological samples can be used for metabolomics studies, such as plasma, urine, tissue, and other body fluids. Each provides different information with particular strengths and weaknesses[2]. The selection of sample sources is largely dependent on study questions and sample availability[3]. Research has shown that more subtle biochemical changes occurred in the earliest stages of OA progression, and altered composition or concentrations of synovial fluid (SF) components are directly linked to OA[4]. SF is an ideal specimen for OA studies because the metabolite concentrations in SF can directly reflect the biological process of cartilage and other joint tissues. However, obtaining SF samples is invasive and not practical in a clinical setting. Thus, one of the most frequently used biological samples in OA research is blood, under the assumption that plasma metabolic components can indirectly reflect biological processes in a joint. The assumption has been tested on only a few carbohydrates and pep-

tides/proteins[5,6,7,8]. More comprehensive investigations are needed. Therefore, we undertook this study to systematically examine the relationship between plasma and SF metabolite concentrations in patients with OA.

MATERIALS AND METHODS

Patients. Our study was part of the Newfoundland Osteoarthritis Study[9]. Patients with OA were recruited from those who underwent total knee replacement surgery owing to primary OA between November 2011 and December 2013 in St. Clare's Mercy Hospital and Health Science Centre General Hospital in St. John's, the capital of Newfoundland and Labrador, Canada. The study was approved by the Health Research Ethics Authority of Newfoundland and Labrador and written consent was obtained from all the participants. Demographic and medical information was collected by a self-administered general questionnaire with the assistance of research staff if necessary.

Metabolic profiling. Blood samples were collected after at least 8 h of fasting, and plasma was separated from the whole blood using a standard protocol. SF samples were collected during joint surgeries. Prior to knee arthrotomy, a syringe was inserted into the suprapatellar pouch of the knee, and 2–4 ml of SF was aspirated. The samples were then put in vials and stored in liquid nitrogen until analysis. Plasma samples were separated from the whole blood samples using a standard protocol and the SF samples were processed based on the instruction of the metabolomics assay kit (Biocrates Life Sciences AG). Metabolic profiling was performed on the Waters XEVO TQ MS system (Waters Ltd.) using the Biocrates AbsoluteIDQ p180 kit, which measures 186 metabolites including 90 glycerophospholipids, 40 acylcarnitines (1 free carnitine), 21 amino acids, 19 biogenic amines, 15 sphingolipids, and 1 hexose (> 90% is glucose). The details of the 186 metabolites are listed in Supplementary Table 1, available online at jrheum.org. The metabolic profiling method using this kit has been described[1].

Statistical methods. Metabolite concentrations in plasma and SF samples were obtained. The metabolites present in at least 80% of samples were used for analysis[10] and the missing values were replaced by half of the minimum value found in the dataset[11]. The ratios of the pairwise metabolite concentrations (168 × 167 = 28,056 ratios) as proxies for enzymatic reaction rates were calculated. Spearman's rank correlation coefficient (ρ) was used to determine the relationship for each metabolite concentration and metabolite ratio between plasma and SF. Statistical analysis was carried out using PCIT package implemented in R (version 3.1.1 for Windows). Bonferroni method was used to correct multiple testing. Significance level was defined as p value $\leq 2.98 \times 10^{-4}$ (0.05/168) for metabolites and p value $\leq 1.78 \times 10^{-6}$ (0.05/28056) for metabolite ratios to control type I error. The correlation analysis was also performed in men and women, separately, as well as people younger and older than 65 years. The results were also confirmed by split-analysis.

RESULTS

A total of 30 male and 39 female patients with knee OA were included in the study. Mean age of participants was 66 ± 8 years, and the mean body mass index was 33.3 ± 6.9 kg/m^2.

Over 90% of the targeted metabolites (168/186) were successfully determined in each sample. These included 40 acylcarnitines (including free carnitine), 20 amino acids, 9 biogenic amines, 87 glycerophospholipids, 11 sphingolipids, and 1 hexose (> 90% glucose).

For each of the 168 metabolites, the Spearman's rank correlation coefficient (ρ) between plasma and SF ranged from –0.12 to 0.59, and 90% of the relationships were positive. The average ρ for all relationships was 0.23 ± 0.13. Only 8 out of 168 metabolites had $\rho \geq 0.45$ with $p \leq 2.98 \times 10^{-4}$. These 8 metabolites were 4 amino acids (glycine, leucine, valine, and isoleucine), 3 glycerophospholipids (phosphatidylcholine diacyl C36:0, phosphatidylcholine acyl-alkyl C30:0, phosphatidylcholine diacyl C24:0), and creatinine (Figure 1). The ρ for all the metabolite concentrations is provided in Supplementary Table 2, available online at jrheum.org. When we examined the correlation separately for men and women, we found that on average men tended to have a higher ρ (0.32 ± 0.25) than women (0.21 ± 0.16), but this difference was not statistically significant. Similarly, there was no difference in ρ between people younger and older than 65 years, and the average ρ was 0.22 ± 0.16 and 0.23 ± 0.17, respectively.

For the 28,056 (168 × 167) pairwise metabolite concentration ratios, ρ ranged from –0.33 to 0.85. The average ρ was 0.29 ± 0.20, and 92% were positive. There were 4018 metabolite ratios with $\rho \geq 0.52$ and $p < 1.78 \times 10^{-6}$, of which 66 metabolite ratios had $\rho \geq 0.8$ (Table 1). The ρ for all the metabolite ratios is provided in Supplementary Table 3, available online at jrheum.org. Similar correlations were observed for men (mean ρ = 0.27 ± 0.27) and women (mean ρ = 0.29 ± 0.20), as well as for people younger and older than 65 years (mean ρ = 0.27 ± 0.21 and 0.29 ± 0.23, respectively).

Split-half analyses in which the whole sample was randomly divided into 2 parts were used to confirm the results. For the 168 absolute concentrations, the average ρ values in 2 parts were 0.20 ± 0.13 and 0.19 ± 0.17, respectively. For the 28,056 metabolite ratios, the average ρ values in 2 parts were 0.25 ± 0.26 and 0.30 ± 0.20, respectively.

Metabolite ratios had consistent correlations in 2 subsamples whereas large discrepancies were observed for some of the metabolite concentrations. For 4018 significant metabolite ratios, the average absolute difference in ρ values between 2 subsamples was 0.13, and 86% of the metabolite ratios had a difference of < 0.25. For the 8 significant metabolites, the average absolute difference in ρ values between 2 subsamples was 0.29. The most consistent correlation was for isoleucine with virtually the same ρ in 2 subsamples. Creatinine was the one with the largest discrepancy, with a ρ of 0.6 in the first half samples and no correlation in the other half samples.

DISCUSSION

Although blood plasma samples have been used in OA research under the assumption that plasma components could indirectly reflect joint biological processes[12,13], to the best of our knowledge, this is the first study that systematically examined the relationship between plasma and SF metabolite concentrations in patients with OA.

Our results documented that all 168 metabolites examined can be detected in both blood plasma and SF, adding evidence to support the assumption. However, we found that metabolite concentrations in plasma and SF vary substantially. The

Table 1. Highly correlated metabolite ratios with ρ ≥ 0.8 (n = 66).

Metabolites		Metabolite Ratios (mean ± SD)		ρ
Numerator	Denominator	Synovial	Plasma	
PC aa C36:3	PC aa C38:5	2.12 ± 0.44	2.43 ± 0.58	0.85
PC aa C38:5	PC aa C36:3	0.49 ± 0.10	0.43 ± 0.11	0.85
PC aa C34:2	PC aa C36:4	1.5 ± 0.39	1.74 ± 0.53	0.85
PC aa C36:4	PC aa C34:2	0.71 ± 0.21	0.62 ± 0.19	0.85
PC ae C38:2	PC ae C44:5	3.34 ± 0.87	3.98 ± 1.31	0.84
PC ae C44:5	PC ae C38:2	0.31 ± 0.08	0.27 ± 0.08	0.84
PC aa C38:4	PC ae C36:2	18.89 ± 5.62	15.12 ± 6.08	0.84
PC ae C36:2	PC aa C38:4	0.05 ± 0.01	0.07 ± 0.02	0.84
SM (OH) C14:1	SM C16:0	0.03 ± 0.01	0.04 ± 0.01	0.84
SM C16:0	SM (OH) C14:1	26.32 ± 5.79	25.44 ± 5.32	0.84
PC ae C38:2	PC ae C38:4	0.42 ± 0.08	0.45 ± 0.09	0.84
PC ae C38:4	PC ae C38:2	2.44 ± 0.52	2.26 ± 0.46	0.84
PC ae C38:3	PC ae C44:5	3.39 ± 0.88	4.31 ± 1.43	0.83
PC ae C44:5	PC ae C38:3	0.31 ± 0.08	0.25 ± 0.08	0.83
PC ae C36:2	PC ae C44:5	4.67 ± 1.29	6.74 ± 2.54	0.83
PC ae C44:5	PC ae C36:2	0.23 ± 0.06	0.16 ± 0.05	0.83
PC ae C36:1	PC ae C44:5	5.35 ± 1.32	6.99 ± 2.39	0.83
PC ae C44:5	PC ae C36:1	0.19 ± 0.05	0.15 ± 0.05	0.83
PC aa C38:4	PC ae C40:6	33.12 ± 7.86	33 ± 11.39	0.82
PC ae C40:6	PC aa C38:4	0.03 ± 0.01	0.03 ± 0.01	0.82
PC aa C36:2	PC aa C38:4	1.42 ± 0.36	1.79 ± 0.57	0.82
PC aa C38:4	PC aa C36:2	0.75 ± 0.21	0.61 ± 0.19	0.82
PC aa C36:4	PC ae C36:2	23.84 ± 7.76	27.09 ± 10.29	0.82
PC ae C36:2	PC aa C36:4	0.04 ± 0.01	0.04 ± 0.01	0.82
PC ae C38:4	PC ae C40:6	3.1 ± 0.56	3 ± 0.59	0.82
PC ae C40:6	PC ae C38:4	0.33 ± 0.06	0.34 ± 0.06	0.82
PC aa C38:4	PC ae C38:1	27.11 ± 6.59	26.9 ± 8.7	0.82
PC ae C38:1	PC aa C38:4	0.03 ± 0.01	0.04 ± 0.01	0.82
PC aa C38:3	PC aa C38:5	1.22 ± 0.27	1.1 ± 0.27	0.82
PC aa C38:5	PC aa C38:3	0.85 ± 0.19	0.96 ± 0.25	0.82
PC ae C38:4	PC ae C44:5	7.92 ± 1.64	8.65 ± 2.10	0.81
PC ae C44:5	PC ae C38:4	0.13 ± 0.02	0.12 ± 0.02	0.81
PC aa C38:6	SM C18:0	2.75 ± 1.15	3.83 ± 1.41	0.81
SM C18:0	PC aa C38:6	0.42 ± 0.17	0.29 ± 0.12	0.81
PC aa C36:4	SM C20:2	160.25 ± 70.35	153.0 ± 67.3	0.81
SM C20:2	PC aa C36:4	0.01 ± 0.01	0.01 ± 0.01	0.81
PC ae C40:6	PC ae C44:5	2.60 ± 0.60	2.97 ± 0.85	0.81
PC ae C44:5	PC ae C40:6	0.4 ± 0.09	0.36 ± 0.10	0.81
PC aa C32:3	PC aa C38:6	0.03 ± 0.01	0.02 ± 0.01	0.81
PC aa C38:6	PC aa C32:3	31.6 ± 15.32	45 ± 20.71	0.81
PC ae C38:5	PC ae C44:5	12.16 ± 2.41	11.96 ± 3.07	0.81
PC ae C44:5	PC ae C38:5	0.08 ± 0.01	0.08 ± 0.02	0.81
PC ae C38:1	PC ae C44:5	3.2 ± 0.82	3.64 ± 1.19	0.81
PC ae C44:5	PC ae C38:1	0.33 ± 0.08	0.3 ± 0.09	0.81
SM (OH) C14:1	SM C16:1	0.26 ± 0.05	0.28 ± 0.05	0.81
SM C16:1	SM (OH) C14:1	3.92 ± 0.80	3.67 ± 0.75	0.81
PC aa C38:4	PC aa C38:6	2.95 ± 1.01	1.86 ± 0.72	0.81
PC aa C38:6	PC aa C38:4	0.37 ± 0.13	0.61 ± 0.23	0.81
PC aa C36:3	PC aa C36:4	0.65 ± 0.16	0.61 ± 0.18	0.81
PC aa C36:4	PC aa C36:3	1.62 ± 0.42	1.76 ± 0.54	0.81
PC aa C38:4	SM C20:2	127.38 ± 54.94	86.14 ± 43.99	0.81
SM C20:2	PC aa C38:4	0.01 ± 0.01	0.01 ± 0.01	0.81
PC ae C38:3	PC aa C38:4	0.43 ± 0.08	0.49 ± 0.10	0.81
PC ae C38:4	PC ae C38:3	2.4 ± 0.48	2.09 ± 0.42	0.81
PC aa C36:2	PC aa C36:4	1.14 ± 0.32	0.99 ± 0.31	0.80
PC aa C36:4	PC aa C36:2	0.95 ± 0.30	1.11 ± 0.38	0.80
PC ae C40:5	PC ae C44:5	2.38 ± 0.41	2.44 ± 0.49	0.80
PC ae C44:5	PC ae C40:5	0.43 ± 0.07	0.42 ± 0.08	0.80
PC aa C38:4	PC ae C32:1	35.4 ± 11.68	54.06 ± 19.91	0.80
PC ae C32:1	PC aa C38:4	0.03 ± 0.01	0.02 ± 0.01	0.80
PC aa C38:4	PC aa C40:6	5.87 ± 1.64	4.87 ± 1.52	0.80
PC aa C40:6	PC aa C38:4	0.18 ± 0.05	0.22 ± 0.07	0.80
PC aa C38:4	PC ae C38:2	26.04 ± 6.86	24.71 ± 8.24	0.80
PC ae C38:2	PC aa C38:4	0.04 ± 0.01	0.04 ± 0.01	0.80
PC aa C38:5	PC ae C44:5	32.57 ± 9.83	43.01 ± 16.73	0.80
PC ae C44:5	PC aa C38:5	0.03 ± 0.01	0.02 ± 0.01	0.80

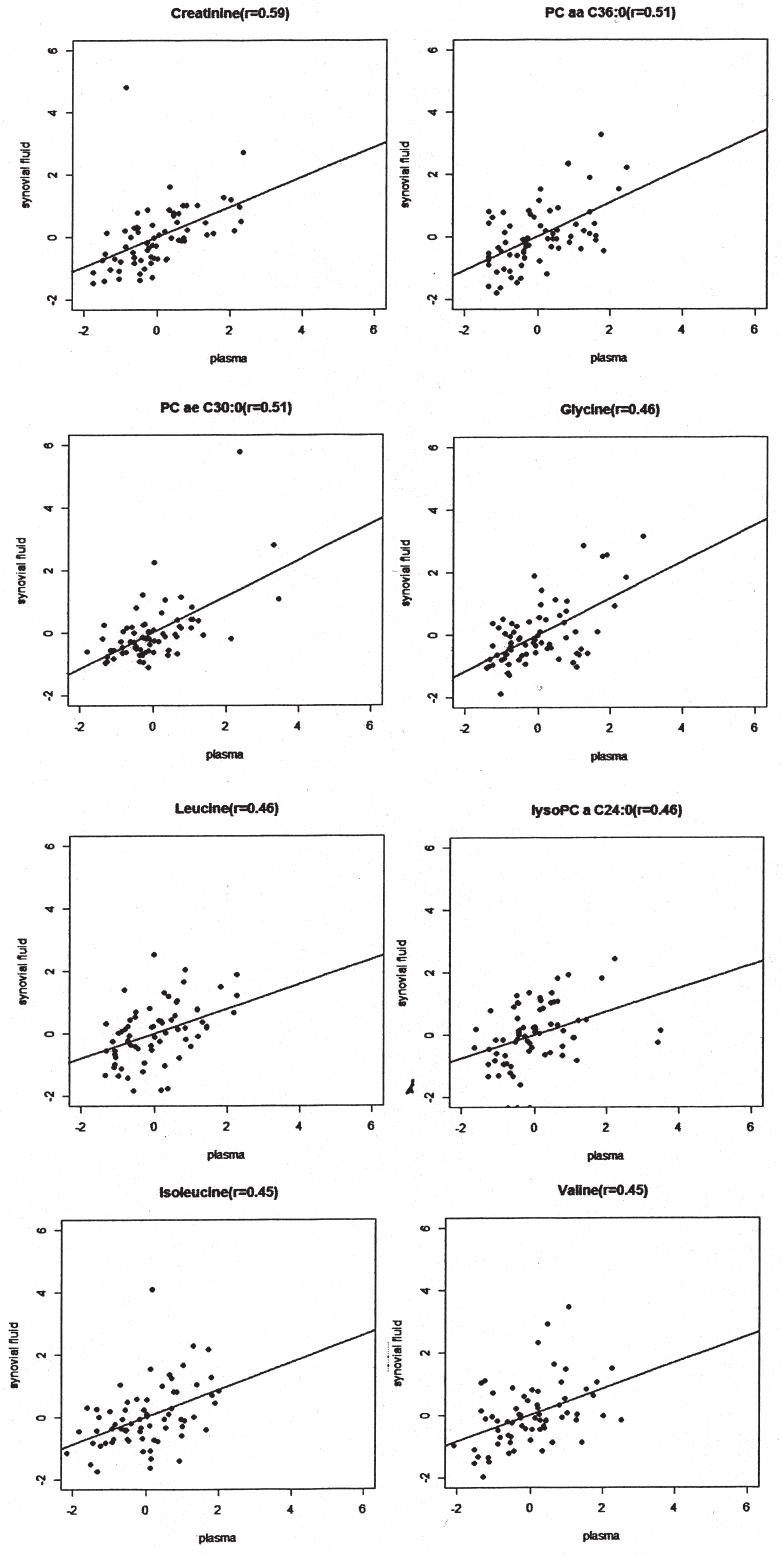

Figure 1. Scatter plots for 8 metabolites with $\rho \geq 0.45$. X axes and Y axes are metabolite concentration Z-scores in plasma and synovial fluid, respectively. PC: phosphatidylcholine.

Table 2. The ρ and key pathways for each category of metabolites.

Metabolites	ρ	Key Pathways
Amino acids (20)	0.29 ± 0.14	Amino acid metabolism, urea-cycle, TCA cycle
Glycerophospholipids (87)	0.26 ± 0.10	Lipid metabolism
Biogenic amines (9)	0.26 ± 0.17	Urea-cycle, amino acid metabolism
Sphingolipids (11)	0.25 ± 0.09	Lipid metabolism
Acylcarnitines (40)	0.12 ± 0.14	Energy metabolism, lipid metabolism
Hexose	0.1	Carbohydrate metabolism
Glycerophospholipids ratios, sphingolipids ratios, amino acid ratios	0.6–0.85	
Biogenic amines, mixed ratios	0.3–0.6	
Carnitines, mixed ratios	< 0.3	

TCA: tricarboxylic acid.

correlation in the metabolite concentrations between plasma and SF is modest, with an average ρ of 0.23, and only 8 out of 168 metabolites were significantly correlated with ρ > 0.45, raising the question as to whether metabolite levels in plasma can reflect joint metabolism for the purpose of monitoring disease progression. Proteomic analyses of SF and plasma in patients with juvenile arthritis showed similar results in which a defined subset of 30 proteins had statistically significant differences between SF and plasma, and distinctive synovial but not plasma proteome expression patterns separate out among patient subgroups[14]. Similarly, Catterall, *et al*[15] measured 12 biomarkers in both serum and SF of patients with acute knee injury. They found that only 4 biomarkers had a significant correlation, with correlation coefficients ranging from 0.5 to 0.8. Cartilage oligomeric matrix protein (COMP) is one of the most studied OA biomarkers, and increased serum COMP levels have been associated with OA. However, serum COMP levels were not correlated with synovial COMP levels in patients with knee OA (r = 0.005)[16].

The mean ρ and key pathways for each category of metabolites are shown in Table 2. Metabolites that show a strong correlation between blood and SF may suggest systemic pathways, whereas metabolites that show low correlations but higher concentrations in SF than in blood would suggest localized metabolism, thus reflecting a joint-specific process. For example, the metabolites arginine and acetylornithine had lower ρ values (0.10 and 0.29) but their concentrations were higher in SF than in plasma by 2.5-fold and 1.9-fold, respectively. Arginine is a crucial factor for bone creation because, among other things, it supports the production of collagen[17]. Arginine deficiency can be the cause of osteoporosis, especially in the case of older women[18]. Adams, *et al*[13] found in synovial tissue that acetylornithine concentration was increased significantly in media with endstage OA versus early/no OA. Therefore, those metabolites need to be studied using joint tissues rather than blood.

However, we found a number of metabolite ratios having a high correlation (ρ ≥ 0.52, 4018 ratios) between plasma and SF. When a pair of metabolites is related to the direct substrates and products of an enzymatic conversion, respectively, the ratio between their concentrations can be used as an approximation of the enzymatic activity[19].

Using metabolite ratios reduced the variance and yielded robust statistical power in genetic association studies[20]. Our previous study found that branched-chain amino acid-to-histidine ratio in serum was associated with knee OA[1]. The correlation of branched-chain amino acid-to-histidine ratio between plasma and SF was 0.56. The results of the current study suggested that those highly correlated metabolite ratios in plasma could serve as surrogates for SF metabolism, and could be used for identifying novel biomarkers and pathways involved in OA development.

Glycerophospholipids form the essential lipid bilayer of all biological membranes and are intimately involved in signal transduction, regulation of membrane trafficking, and many other membrane-related phenomena[21,22]. A study by Hills indicates that alterations in phospholipid composition and concentrations are associated with the development of OA[23]. Phospholipids and enzymes involved in lipid metabolism were demonstrated to affect the regulation of the signaling steps leading to neutrophil activation[24,25]. In addition, lysophosphatidylcholine (LPC) plays an important role in inflammation[26]. LPC is generated by the hydrolysis of membrane PC by phospholipase A2, acts as a chemoattractant at the sites of inflammation, and promotes the inflammatory reaction. Fuchs, *et al*[27] showed the PC/LPC ratio detected in plasma may serve as an indicator of rheumatic arthritis in early stages. In our current study, we found 70 PC/LPC ratios had ρ ≥ 0.52.

Carnitine and its acyl esters acylcarnitines are essential compounds for the metabolism of fatty acids. Carnitine acyltransferases are responsible for the production of acylcarnitines, and the value of acylcarnitines/carnitine ratio can reflect its activity[19,28]. Carnitine can assist the transport and metabolism of fatty acyl-CoA from the cytosol to the mitochondrial matrix, where the enzymes of β-oxidation are located and fatty acids are oxidized as a major source of

energy. Based on the ratio of acylcarnitine to carnitine in SF, the patients with OA could be divided into 2 distinct groups[29]. However, the correlation of the ratio between SF and plasma was low, suggesting it is joint specific.

There are some caveats. First, all our study participants were endstage patients with OA, thus the findings might not be readily generalized to patients with early OA or the general population. Second, anesthesia as a normal practice in the surgery could influence vascular and lymphatic permeability and therefore maybe affect the results obtained. Third, inflammation could have an effect on vascular permeability and the observed results. However, the current study is a paired study, e.g., SF and plasma samples were collected from the same individuals. Inflammation would influence the majority, if not all, of the metabolite concentrations in SF and plasma; thus we would expect to observe a similar correlation for the majority of the metabolite and metabolite ratios, which in fact is not the case in our results, suggesting this is not a big concern. Last, while split-half analyses showed individual correlations for metabolite ratios were stable and consistent between 2 subsamples, a large discrepancy between 2 subsamples was observed for some of the metabolite concentrations, limiting their generalizability to other populations. Further studies are required to confirm the results, particularly for metabolite concentrations.

Correlation between blood plasma and SF metabolite concentrations are modest. Metabolite ratios, which are considered proxies for enzymatic reaction rates and have high correlations, should be considered when using blood plasma as a surrogate of SF in OA biomarker identification.

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ONLINE SUPPLEMENT

Supplementary data for this article are available online at jrheum.org.

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