Supporting Information

Biomarkers of early stage osteoarthritis, rheumatoid arthritis and musculoskeletal health

Running title: Citrullination and differential autoimmunity in early arthritis

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Supporting text S1 | Effect of drug therapy on CP and free hydroxyproline. The effect of drug therapy on CP and Hyp in patients with aRA was evaluated. Patients receiving anti-TNFα therapy had lower plasma Hyp with respect to those not receiving anti-TNFα therapy (0.96 μM versus 3.37 μM, $P<0.01$). Patients receiving treatment with non-steroidal anti-inflammatory drugs (NSAIDs) had lower synovial fluid CP (0.15 versus 1.02 mmol/mol arg, $P<0.05$) and plasma Hyp (0.99 μM versus 3.16 μM, $P<0.01$) with respect to those not receiving NSAIDs. However, patients receiving treatment with prednisolone had higher synovial fluid CP (1.72 versus 0.24 mmol/mol arg, $P<0.01$) and plasma Hyp (2.87 versus 0.94 μM, $P<0.05$) with respect to those not receiving prednisolone. Treatment with or without methotrexate and opiate analgesics was not associated with differences in these variables.

Table S1 | Analytical variables and assay characteristics for quantitation of citrulline, arginine and 4-hydroxyproline by stable isotopic dilution analysis tandem mass spectrometry.

Analyte	Citrulline	Arginine	4-Hydroxyproline
Retention time R_t (min)	13.5	16.5	5.6
Molecular ion M+1 (Da)	176.1	175.1	132.0
Fragment ion (Da)	70.1	70.1	86.1
Cone voltage (V)	20	30	26
Collision energy (eV)	21.0	24.0	12.0
Neutral fragment losses	H_2CO_2, $NH_2C(=O)NH_2$	H_2CO_2, $NH_2C(=NH)NH_2$	H_2CO_2
Internal standard	[5-^{13}C,4,4,5,5-$^{2}H_4$]citrulline	[guanidino-$^{15}N_2$]arg	4,5-[$^{13}C_2$]Hydroxyproline
Limit of detection (fmol)	62	520	102
Intra- and interbatch CV (%; n = 6)	1.3 and 6.0	1.0 and 1.8	1.0 and 1.5
Recovery (%)	88 (protein digest)	94 (protein digest)	100 (ultrafiltrate)

Table S2 | Cross validation of multiclass algorithm. Improved outcome with training set and test set cohorts combined using GLMNET algorithm and randomly assigning 67% samples to training set and 33% to the test set.

A. Multi-class analysis for GLMNET algorithm

	Control	eOA	eRA	non-RA
nCorrect	13/17	10/14	10/15	7/15
Sensitivity	0.76 (0.50 – 0.93)	0.71 (0.42 – 0.92)	0.67 (0.38 – 0.88)	0.47 (0.21 – 0.72)
Specificity	0.77 (0.62 – 0.89)	0.96 (0.85 – 0.99)	1.00 (0.92 – 1.00)	0.80 (0.66 – 0.91)
F-Measure	0.65	0.77	0.80	0.45

B. Confusion matrix for GLMNET algorithm

		Predicted class			
		Control	Early OA	Early RA	non-RA
Clinical	Control	13	2	0	8
class	eOA	2	10	0	0
	eRA	0	0	10	0
	non-RA	2	2	5	7

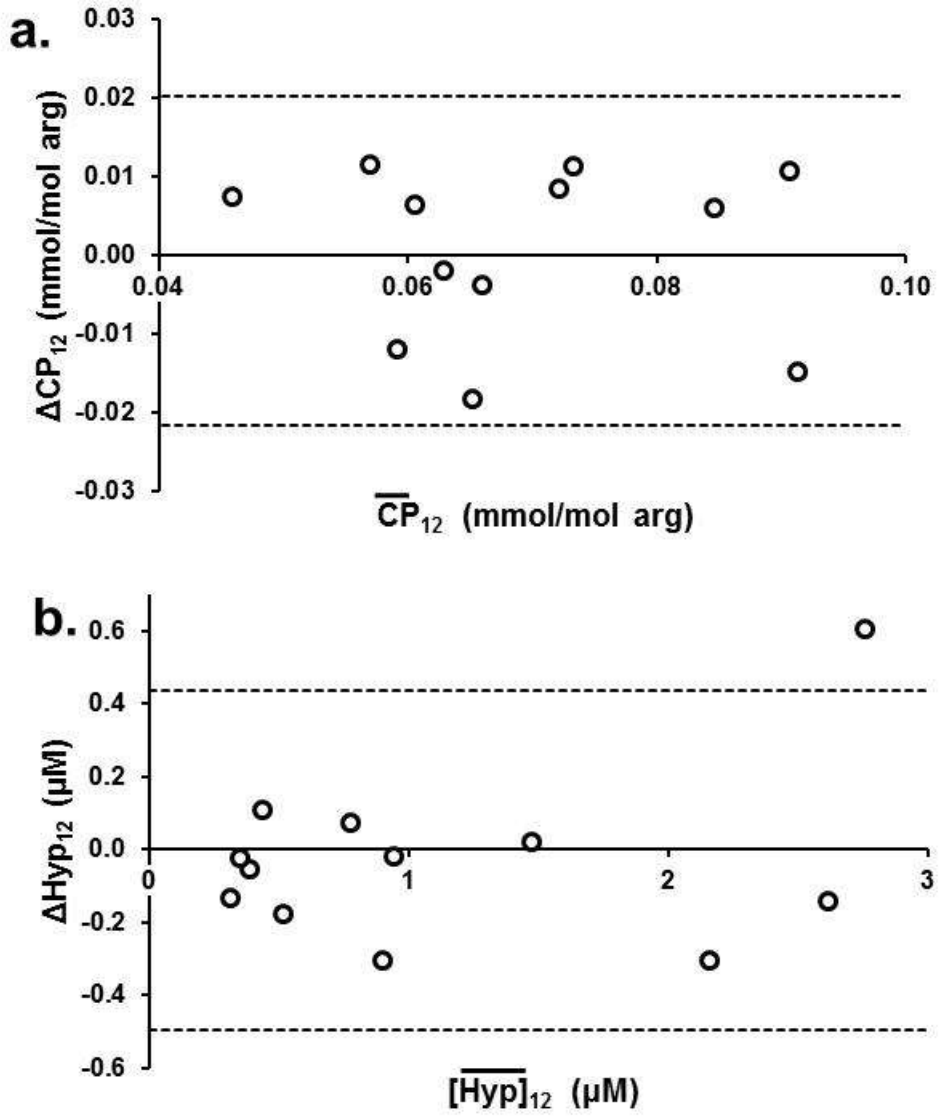

Figure S1 | Bland-Altman plots for (a) CP and (b) Hyp measurements. Data from samples analysis of 12 subjects (7 healthy controls and 5 eOA) was evaluated. There was no proportional bias but one outlier was identified in the Hyp measurements.