Dietary Caffeine Intake Does Not Affect Methotrexate Efficacy in Patients with Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** Methylxanthines, like caffeine, have been thought to reverse the antiinflammatory effects of methotrexate (MTX) in rheumatoid arthritis (RA). We investigated whether patients with RA taking MTX with a higher dietary caffeine intake have a worse clinical response to MTX than those with a lower intake.

Methods. Patients with RA enrolled in a prospective cohort study and currently taking MTX were divided equally into low, moderate, and high caffeine consumers. MTX clinical response was defined by the Disease Activity Score (DAS)28, Multidimensional Health Assessment Questionnaire (MDHAQ) score, and duration of morning stiffness. Regression models were used to study the relationship between caffeine intake and MTX response adjusting for age, sex, and other relevant variables at study enrollment.

Results. Two hundred and sixty-four patients with RA taking MTX had an average caffeine intake of 211.7 mg and average MTX dose of 16.0 mg/wk. The low caffeine group comprised 87 patients, the moderate 86, and the high 91. In 3 multivariate models, there was no statistical difference in MTX efficacy between groups, as measured by DAS28 score, MDHAQ score, and duration of morning stiffness at study enrollment. Moderate and high caffeine group had higher DAS28 scores, physician's global assessment, and swollen joint counts, but differences were not significant.

Conclusion. Caffeine intake among patients taking high doses of MTX for RA did not affect MTX efficacy and RA disease activity over time. (J Rheumatol 2006;33:1275–81)

Key Indexing Terms:
RHEUMATOID ARTHRITIS CAFFEINE METHOTREXATE AICAR ADENOSINE

Methotrexate (MTX) is the most commonly prescribed disease modifying antirheumatic drug (DMARD) for rheumatoid arthritis (RA)[1]. Originally developed as an antimetabolite, it is now given in doses of 7.5 mg to 25 mg per week for RA, psoriasis, and other rheumatic illnesses[2]. Several studies published over the last few years have postulated that caffeine consumption may interfere with the efficacy of MTX[3-6]. While high doses of caffeine administered to rats treated with MTX appeared to reverse the benefits of the medication, results have not been validated in large populations of human subjects[3].

The biological mechanism by which MTX acts in RA is complex, and no single theory explains all of its effects. MTX, which has a structure similar to folic and folinic acid, inhibits multiple folate-dependent metabolic processes. One interesting effect, accorded increasing importance in the literature, is its impact on the adenosine cascade. Patients treated with MTX have an increased concentration of the purine nucleoside, adenosine, in their blood and urine[7]. MTX, an antifolate prodrug that is converted to polyglutamates in the body's cells, likely increases levels of adenosine by blocking a step in purine biosynthesis, leading to the accumulation of its intermediates[8]. MTX polyglutamates inhibit the enzyme 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) transformylase, raising intracellular levels of AICAR, which in turn increases levels of adenosine[9]. It has also been proposed that MTX contributes to the dephosphorylation of adenine nucleotides, creating an accumulation of adenosine in the tissues. Adenosine exerts potent antiinflammatory action by inhibiting inflammatory cell function and production of inflammatory cytokines, largely through receptors on the surface of neutrophils, macrophages, and endothelial cells[8,10-12].

Methylxanthines, such as the asthma drug theophylline or the caffeine found in tea, coffee, some soft drinks, cocoa, and chocolate, are adenosine receptor antagonists[13]. Since MTX likely acts by increasing extracellular adenosine, methylxanthines, in preventing the nucleoside from acting on receptors, could reverse the effects of MTX therapy. In a rat adjuvant model of inflammatory arthritis, caffeine and theophylline attenuated the antiinflammatory benefits of MTX[3]. In 2001, in the first investigation of potential antagonistic action of caffeine in humans treated with MTX, Silke and colleagues showed that, in a cohort of 91 patients with RA, of those who stopped taking MTX, 26% were regular coffee drinkers, compared with only 2% of patients still receiving MTX. Eighty

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percent of patients who had been heavy coffee drinkers discontinued MTX due to treatment failure, leading to the hypothesis that caffeine interfered with MTX efficacy[4]. In a subsequent study of 39 patients with RA, Nesher, *et al* presented additional evidence that caffeine could reverse some benefits of MTX therapy in patients with RA[5].

Previous studies on effects of caffeine on MTX efficacy have not addressed differences in dose duration and size, timing of caffeine ingestion, or the possibility of a threshold level of caffeine required to reverse the antiinflammatory mechanism. MTX is polyglutamated and retained intracellularly for long periods. Its half life is 8 hours, but the clinical effects last for a week[14]. Adenosine receptors, overstimulated by increased adenosine in and around cells, may become desensitized by MTX use over time, potentially modifying the effect methylxanthines could exert. With G-protein-coupled receptors, such as those activated by adenosine, repeated agonist exposure has been shown to result in down-regulation of receptors[15,16]. In addition, research has shown that receptors may function differently in chronic and acute inflammation, indicating that perhaps longer studies are needed to determine the effect of caffeine on chronic, systemic disease[17].

We investigated in a large cohort with RA whether patients taking MTX who reported a higher dietary caffeine intake had a worse clinical response to MTX than those reporting a lower caffeine intake. We examined and compared inflammatory changes in the 3 groups at study enrollment.

MATERIALS AND METHODS

Study population. We studied a sample of 264 patients currently receiving MTX therapy drawn from the Brigham Rheumatoid Arthritis Sequential Study (BRASS). BRASS is a prospective observational cohort of 900 patients with rheumatologist-diagnosed RA that seeks to identify biomarkers and genetic indicators to predict drug response and toxicity and disease activity in patients with RA. All patients at the Robert Breck Brigham Arthritis Center with a billing diagnosis of RA (714.0) or seronegative inflammatory arthritis (714.9) over 18 years old without systemic lupus erythematosus or psoriatic arthritis were eligible for recruitment.

The study collected information on disease severity, functional status, level of fatigue, medications, and adverse events. At enrollment, patients completed self-administered and interview questionnaires describing demographic information, functional status, and general health state; they provided hand radiographs and serum, DNA, and RNA for proteomic and genetic analysis. Every 6 months they completed self-administered questionnaires through the mail, updating medications, general health state and functional status. At yearly clinic visits, patients provided RNA and serum, as well as additional interview and self-administered questionnaires. Physicians completed 28-swollen joint counts, global assessments, and information about comorbidities at baseline and every year thereafter.

All research was carried out in compliance with the Helsinki Declaration and approved by the Brigham and Women's Hospital Internal Review Board.

Exposure. At enrollment, all BRASS participants completed a food frequency questionnaire (FFQ) providing information about their average consumption of beverages over the past year. They described how often and in what quantities they consumed caffeinated colas, both diet and regular; other carbonated beverages with caffeine, such as Dr. Pepper; coffee, both decaffeinated and regular; and tea, also both decaffeinated and regular. Patients were not asked about intake of chocolate or other foods with caffeine.

We calculated the amount of caffeine in each type of drink using values provided by the Harvard Nurses Health Study, obtained from the US Department of Agriculture[18]. We determined daily caffeine intake (in mg) for each participant by multiplying the frequency of consumption of each beverage unit (one cup of tea or coffee and one can of cola) by the caffeine content of the specified unit. We divided the patients taking MTX into 3 groups: low, moderate, and high caffeine intake. We chose to separate the cohort into 3 groups to better capture differences along the Gaussian distribution through a larger separation of the low and high groups.

Only one patient in the cohort reported taking Uniphyl (a sustained release form of theophylline).

Outcome. Our primary outcome for MTX clinical response was the Disease Activity Score-28 (DAS28), calculated from data collected at enrollment; we used components of the DAS score [C-reactive protein (CRP) level, Physician's Global Assessment (PGA) of disease severity, and the 28-swollen joint count (SJC)] as secondary measures to evaluate whether one factor was driving the outcome. As additional secondary outcomes, we evaluated duration of morning stiffness and the Multidimensional Health Assessment Questionnaire (MDHAQ) score to assess functional capabilities and extent of current disease activity. We defined each variable categorically based on generally accepted cut-off points. DAS28 low: < 3.2, DAS28 high: ≥ 3.2; CRP low: < 3.0, CRP high: ≥ 3.0 high; PGA low: < 4.0, PGA high: ≥ 4.0; SJC low: < 6.0, SJC high: ≥ 6.0; morning stiffness low: < 1 hour, high: ≥ 1 hour; MDHAQ low: < 1.0, MDHAQ high: ≥ 1.0.

Statistical analyses. We compared the 3 caffeine-intake groups across a series of variables, including age, gender, marital status, educational level, annual income, disease duration, extent of fatigue, and MTX dose. We presented p values from t tests for continuous variables (age, RA disease duration, fatigue, and MTX dose) and chi-square analyses for the categorical exposures (all others). To compare the 3 groups at enrollment, we used a crude model and 2 multivariate models, adjusting for RA duration and additionally adjusting for age and sex. We used a significance level of 0.05 for all p values and 95% confidence intervals (CI) for all models. All analysis was performed using SAS statistical package version 9.1.

RESULTS

Population characteristics. The cohort had a mean age of 57.4 years (83.3% women) and mean disease duration of 14.4 years. Average caffeine intake was 211.7 mg daily, slightly more than the average daily intake for the American adult, estimated in recent research at 200 mg (about one and a half cups of coffee)[19,20]. Average MTX dose was 16.0 mg/wk. We analyzed 87 low caffeine consumers with a mean age of 58.3 years (± 15.2), 86 moderate caffeine consumers with a mean age of 58.3 years (± 14.1), and 91 high caffeine consumers with a mean age of 55.6 years (± 13.3) (p = 0.33) (Table 1). Differences between the 3 groups in gender, age, and annual income were not statistically significant. Eighty-four percent of those in the low caffeine group were female, compared with 86% in the moderate intake group, and 80% in the high intake group (p = 0.57). There were marginally significant differences in educational levels between the groups, with a greater percentage of the moderate caffeine consumers achieving the highest level of education (p = 0.05) (Table 1).

While we hypothesized that people with a higher caffeine intake could also have a higher level of fatigue, no statistically significant difference between the groups was observed, as measured by the visual analog scale (p = 0.17). Mean MTX dose was similar at 17.1 mg (± 5.8) for the low caffeine consumers, 15.4 mg (± 5.7) for the moderate consumers, and 15.5

Table 1. Socio-demographic and clinical characteristics of our study patients according to caffeine consumption. Significance was determined using analysis of variance for continuous variables and chi-squared analysis for categorical variables.

Variable	Low Intake n = 87	Moderate Intake n = 86	High Intake n = 91	p
Caffeine mg/day, median (range)	39 (0–105)	165 (106–260)	422 (260–1058)	
Age, mean ± SD	58.3 ± 15.2	58.3 ± 14.1	55.6 ± 13.3	0.33
Sex, n (% female)	73 (84)	74 (86)	73 (80)	0.57
Marital status, n (% married)	52 (60)	54 (63)	58 (64)	0.85
Educational level, n (%)				0.05
High shool	20 (23)	25 (29)	17 (19)	
College	45 (52)	33 (38)	56 (62)	
Graduate school	22 (25)	28 (33)	18 (20)	
Annual income, n (%)				0.65
< $30,000	17 (25)	17 (23)	12 (15)	
$30,000–69,999	23 (33)	25 (34)	30 (38)	
≥ $70,000	29 (42)	32 (43)	38 (47)	
Disease duration, n (% ≥ 10 yrs)	53 (62)	57 (66)	47 (52)	0.12
Fatigue, VAS 0–100, median	50.0	42.5	40.0	0.17*
MTX dose, mean ± SD	17.1 ± 5.8	15.4 ± 5.7	15.5 ± 5.2	0.08
MTX duration, mo, median	42.0	48.0	30.0	0.35*
Number of current DMARD, n (%)				0.29
0	35 (40)	44 (51)	46 (51)	
1	45 (52)	37 (43)	43 (47)	
2	7 (8)	5 (6)	2 (2)	
Number of past DMARD, n (%)				0.16
0	24 (28)	17 (20)	26 (29)	
1	14 (16)	20 (23)	28 (31)	
2	22 (25)	23 (27)	14 (15)	
≥ 3	27 (31)	26 (30)	23 (25)	

* Wilcoxon Rank Sum test. VAS: visual analog scale; MTX: methotrexate; DMARD: disease modifying antirheumatic drug.

(± 5.2) for the high consumers (p = 0.08). Those in the moderate group had a longer average disease duration, with 66% having been diagnosed with RA for 10 years or more, while only 52% of those in the highest group and 62% of those in the low group reported disease duration of 10 years or greater. None of the differences were significant (p = 0.12) (Table 1). There were no significant differences between the 3 groups in duration of MTX use (p = 0.35) or in the number of DMARD taken (Table 1).

Relationship of caffeine intake with different disease severity variables. Disease activity as measured by DAS28, CRP, PGA, SJC, morning stiffness, and MDHAQ, did not vary significantly between groups. While those with moderate and high caffeine consumption generally had more elevated DAS28, PGA, and SJC, the differences were not statistically significant (Table 2). DAS28 levels did not differ significantly between the 3 groups in any of the models [in the fully adjusted model; for moderate caffeine intake, odds ratio (OR) = 1.6, 95% CI: 0.8–3.2; for high caffeine intake, OR = 1.6 95% CI: 0.8–3.4]. There was also no significant change in CRP levels between the 3 groups. Similarly, differences in duration of morning stiffness and MDHAQ scores were statistically insignificant (Table 2). Six percent of the MTX users had been taking the medication for 2 months or less, but adjusting for MTX duration made no difference in the multivariate model (results not shown). Figure 1 depicts the graphs of the adjusted OR for these results.

When we adjusted for any medications that could consistently modify inflammation levels and thus MTX efficacy (prednisone, solumedrol, sulfasalazine, leflunomide, and tumor necrosis factor (TNF)-α inhibitors), OR for our model remained around 1.0 (results not shown). Of the 264 MTX users, 29.2% were taking folic acid, 26.3% leucovorin, and 51.2% either leucovorin or folic acid. In our model, leucovorin and folic acid consumption did not confound results. In addition, cigarette smoking has been hypothesized to interfere with some antiinflammatory effects of MTX. In our cohort, however, there were only 16 current smokers among the MTX users, and smoking was also not a confounding factor (results not shown).

DISCUSSION

Adenosine acts through 4 subclasses of receptors, known as P1 receptors, found on inflammatory cells: A_1, A_{2A}, A_{2B}, and A_3[21]. These are large transmembrane proteins that influence cell signaling by coupling with G-proteins thus modulating any number of life-sustaining processes[6,22]. There are multiple theories as to how, by acting on these receptors, adenosine

Table 2. Mean and 95% confidence interval (CI) of disease activity measures for the main exposures: moderate versus low and high versus low caffeine dietary intake. RA duration was measured as a continuous variable.

Outcomes	Univariate Models, OR (95% CI)	Multivariate Model 1 Adjusted for Duration of RA, OR (95% CI)	Multivariate Model 2, Further Adjusted for Age and Sex, OR (95% CI)
DAS28 (< 3.2 vs ≥ 3.2)			
Low	1.0	1.0	1.0
Moderate	1.5 (0.7–2.9)	1.6 (0.8–3.2)	1.6 (0.8–3.2)
High	1.3 (0.7–2.5)	1.6 (0.8–3.1)	1.6 (0.8–3.4)
CRP (< 3 vs ≥ 3)			
Low	1.0	1.0	1.0
Moderate	1.5 (0.8–2.9)	1.5 (0.8–2.9)	1.5 (0.8–2.9)
High	0.7 (0.4–1.4)	0.7 (0.4–1.4)	0.7 (0.4–1.5)
PGA score (< 4 vs ≥ 4)			
Low	1.0	1.0	1.0
Moderate	1.4 (0.7–2.8)	1.4 (0.7–2.8)	1.4 (0.7–2.9)
High	1.3 (0.6–2.6)	1.3 (0.7–2.7)	1.3 (0.6–2.6)
28-Swollen joint count (< 6 vs ≥ 6)			
Low	1.0	1.0	1.0
Moderate	1.5 (0.8–2.7)	1.6 (0.9–2.9)	1.6 (0.9–2.9)
High	1.3 (0.7–2.3)	1.4 (0.8–2.7)	1.5 (0.8–2.7)
Morning stiffness (≤ 1 hour vs > 1 hour)			
Low	1.0	1.0	1.0
Moderate	0.6 (0.3–1.3)	0.6 (0.3–1.2)	0.6 (0.3–1.2)
High	1.1 (0.6–2.2)	1.1 (0.5–2.1)	1.1 (0.5–2.1)
MDHAQ (≤ 1 vs > 1)			
Low	1.0	1.0	1.0
Moderate	1.2 (0.6–2.6)	1.4 (0.6–2.9)	1.4 (0.6–2.9)
High	0.9 (0.4–1.9)	1.0 (0.5–2.1)	1.0 (0.4–2.1)

DAS: disease activity score; MDHAQ: multidimentional health assessment questionnaire; PGA: physician's global assessment; OR: odds ratio.

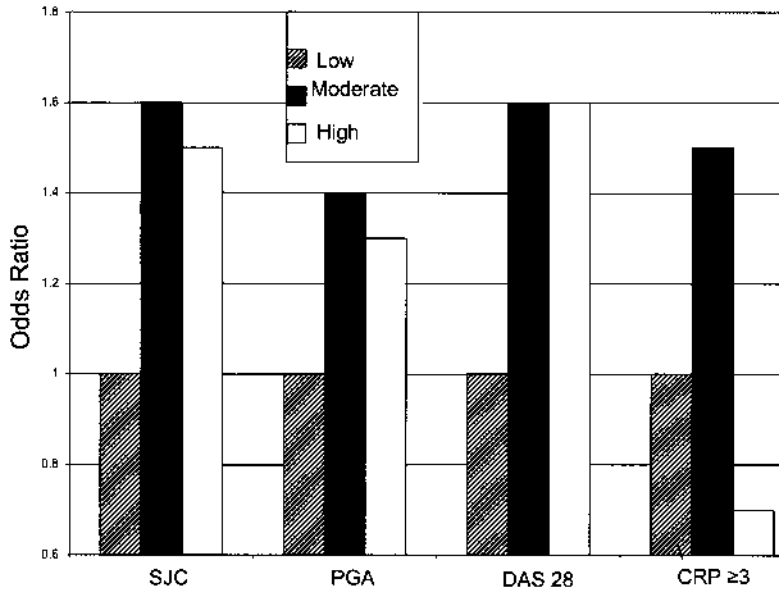

Figure 1. Measures of MTX efficacy for the 3 caffeine-intake groups. *p values were not significant in comparisons across all groups.

suppresses inflammation and protects the body from its effects. First, through the A_{2A} receptor on the neutrophil membrane surface, it may stop the production of the superoxide anion and formation of respiratory burst reactions, protecting vascular endothelial cells from tissue damage[23]. Second, high concentrations of adenosine, through the media-

tion of A_2 receptors, can inhibit Fc-gamma-receptor phagocytic activity of monocytes, although low concentrations of adenosine have the opposite effect. Adenosine also potentially influences cytokine production through TNF-α, interleukin (IL)-6, IL-8, IL-10, and IL-12[24]. Third, adenosine likely inhibits inflammatory action of the endothelial cells by enhancing impermeability of the cell barrier, by encouraging angiogenesis of endothelial cells, and by suppressing production of certain key cytokines and adhesive molecules, such as vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1[25]. Finally, again through the A_2 receptors, adenosine may be involved in T cell deactivation and apoptosis of certain T cells[6,10].

Methylxanthines non-selectively antagonize these receptor-mediated processes[26] and thus could be expected to counteract some of the adenosine-mediated beneficial effects of MTX therapy. In a rat adjuvant arthritis model, caffeine or theophylline, administered over a 25-day period at 10 mg/kg/day, to rats receiving 0.75 mg/kg/wk of MTX reversed antiinflammatory actions of the medication. Neither caffeine nor theophylline administered without MTX affected the onset or severity of arthritis in the rats. The authors concluded that their findings provided strong evidence that, in this model of RA, MTX exerts its antiinflammatory effects through adenosine, likely acting through all 4 receptors[3]. Rats in the study, however, received 3–4 times more caffeine per kg/day than the participants in our cohort, who ingested an average of 211.7 mg of caffeine per day. Their doses of caffeine and MTX were carefully regulated, unlike the more natural consumption patterns of our subjects.

Our findings conflict with the results of the aforementioned study and 2 previous studies performed in human populations on the effects of caffeine on MTX efficacy. Both previous human population studies had sample sizes less than half of ours, limiting their capacity for multivariate analysis. The Silke study suggested that caffeine had potentially interfered with MTX efficacy in the 80% of participants who were heavy coffee drinkers. Caffeine intake was defined by the number of cups of coffee, measured on a weekly basis, rather than mg of caffeine, introducing variability of cup size and caffeine content into the analysis[4].

In Nesher's study of patients with RA taking MTX, those who consumed more than 180 mg of caffeine daily experienced less improvement in morning stiffness and joint pain than those who consumed less than 120 mg of caffeine[5]. Across a number of other variables, including tender joint count, swollen joint count, and erythrocyte sedimentation rate (ESR), there was no statistical difference between the caffeine groups. Our study methodology differed from Nesher's in several ways that could explain the discrepancies in the results. First, the study's small sample size of 39 patients limited its statistical power and capacity to infer results. Second, Nesher's participants started receiving MTX at the inception of the study, while many of our patients had been taking the medication for years prior to their enrollment in BRASS. Our study, therefore, may have captured the longterm effects of MTX and caffeine interaction, an area largely unexplored and perhaps affected by desensitization of adenosine receptors. Adjusting for MTX duration within our own caffeine groups, however, did not affect our results. Third, while the average caffeine consumption was similar, patients in Nesher's study were on a lower dose of MTX, starting the study at 7.5 mg of MTX per week, while the average for our study was more than double that number (16.0 mg), meaning that our subjects may have required a higher caffeine consumption to reverse the antiinflammatory effects of the higher dose of MTX[5].

In our cohort of 264 patients, caffeine intake did not appear to affect MTX efficacy. The number of patients in the cohort provided 80% power to detect an OR of 2.6 between the low and high caffeine consumers. We determined DAS28 to be an effective measure of overall disease activity because it includes both serum indicators of inflammation and physician evaluations of disease control. It thus seemed an accurate surrogate for MTX efficacy. We also looked at the 3 components of the DAS independently to determine whether one was independently driving our results. We further examined 2 other clinical measures of disease activity: morning stiffness and MDHAQ. In comparing the 3 categories of caffeine consumers across these variables and adjusting first simply for disease duration and then for age, sex, and disease duration, we did not find statistically significant differences.

We hypothesized that perhaps, in these patients, MTX had a secondary effect. Other medications that did not rely on the adenosine cascade were instead controlling their disease activity. Adjusting our multivariate models for confounding effects of other medications, however, did not change the outcomes. There was also no significant difference between the 3 groups in the total number of DMARD taken. When we adjusted for any medications that could consistently modify inflammation levels and thus MTX efficacy (prednisone, solumedrol, sulfasalazine, leflunomide, and TNF-α inhibitors), OR for our model remained around 1.0 (results not shown). Leucovorin (folinic acid) and folic acid taken to reduce side effects of MTX have been shown to reduce its antiinflammatory benefits in large doses[27-29]. In our model, leucovorin and folic acid consumption use did not confound the results. In addition, cigarette smoking has been hypothesized to interfere with some of the antiinflammatory effects of MTX, such as reduction of the superoxide anion generation[30], but in our cohort smoking was not a confounding factor.

Our study has several limitations. First, the caffeine doses consumed by our study population might not have been high enough to antagonize the effect of MTX, although our caffeine intake is similar to the average caffeine intake in previous studies. Second, our results relied on the accuracy of patient self-reported caffeine use. Each caffeine intake per drink was approximated, based on the average amounts in a cup of coffee, tea, or soda without accounting for differences

across brands or cup sizes. We did not measure caffeine plasma levels to verify patient reports. Third, we converted weekly caffeine consumption into an average daily dose and did not account for the timing of the caffeine consumption either within the day or the week; timing of the MTX dose and caffeine consumption could influence how much effect the adenosine antagonist has on MTX. Fourth, individuals eliminate caffeine from their blood differently and have different factors influencing the caffeine-metabolizing enzyme, CYP1A2, making it difficult to measure caffeine levels across a sample population[31]. Finally, there are other mechanisms that explain the action of MTX in RA that we did not take into account, in particular some proinflammatory action through A_1 receptors[32,33]. Khoa, *et al* found that several prominent Th-1 inflammatory cytokines mediate the effects of A_{2A} adenosine receptors, perhaps indicating that the extent of local inflammatory response may influence receptivity of the RA patient to MTX and its antagonists and thus the effect of caffeine on MTX efficacy[34].

In this cohort of patients with longstanding RA, those in the high caffeine group did not have a worse response to MTX than those in the low or moderate caffeine groups; given our 80% power to detect a large effect, differences in DAS28 and other inflammatory markers could not be detected. We suggest additional large cohort studies of the impact of caffeine on MTX and further work to correlate caffeine ingestion with caffeine plasma levels and MTX efficacy.

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