Methotrexate reduces HbA1c concentration but does not produce chronic accumulation of ZMP in patients with rheumatoid or psoriatic arthritis

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Objectives: The mechanism by which methotrexate (MTX) improves glucose homeostasis in patients with rheumatoid (RA) and psoriatic arthritis (PsA) remains undetermined. Animal studies indicate a role for intracellular accumulation of 5-aminomidazole-4-carboxamide-1-β-d-ribofuranosyl 5′-monophosphate (ZMP) but this has not been directly demonstrated in humans. We explored whether accumulation of ZMP is associated with improvements in glucose homeostasis during MTX therapy.

Method: MTX-naïve, non-diabetic RA (n = 16) and PsA (n = 10) patients received uninterrupted MTX treatment for 6 months. To evaluate whether ZMP accumulated during MTX therapy, we measured the concentration of ZMP in erythrocytes and the concentration of its dephosphorylated derivative 5-aminimidazole-4-carboxamide-1-β-d-ribofuranoside (AICAR) in urine using liquid chromatography mass spectrometry (LC-MS/MS). To assess glucose homeostasis, we determined the concentration of glycated haemoglobin (HbA1c) and homeostasis model assessment of insulin resistance [HOMA-IR: fasting glucose (mmol/L) × fasting insulin (μU/mL)/22.5].

Results: Erythrocyte ZMP and urinary AICAR concentrations did not increase during 6 months of MTX therapy. HbA1c concentration was reduced from 5.80 ± 0.29% at baseline to 5.51 ± 0.32% at 6 months (p < 0.001), while HOMA-IR remained unaltered. Reduction in HbA1c concentration was not associated with increased ZMP or AICAR concentrations.

Conclusions: MTX therapy probably does not produce a chronic increase in erythrocyte ZMP or urinary AICAR concentrations. Collectively, our data do not support the hypothesis that MTX improves glucose homeostasis through chronic accumulation of ZMP.

Methotrexate (MTX) is a first-line disease-modifying anti-rheumatic drug (DMARD) for the treatment of rheumatoid arthritis (RA) and psoriatic arthritis (PsA). Evidence suggests that therapy with MTX might reduce the risk of diabetes mellitus and the metabolic syndrome in patients with RA or PsA (1–3). However, the underlying pharmacological mechanism remains unclear. Mechanisms by which MTX suppresses inflammation are thought to involve an increase in adenosine formation (4). According to animal experiments, this increase results from MTX-induced intracellular accumulation of 5-aminimidazole-4-carboxamide-1-β-d-ribofuranosyl 5′-monophosphate (ZMP) (4). In addition to enhancing adenosine formation, ZMP activates the adenosine monophosphate (AMP)-activated protein kinase (AMPK) (5), a cellular energy sensor and a major regulator of energy metabolism. Pharmacological activation of AMPK ameliorates glucose homeostasis in diabetes mellitus (6, 7). MTX may therefore ameliorate glucose homeostasis by promoting ZMP accumulation and AMPK activation (8, 9).

ZMP is a purine precursor that is normally converted to inosine monophosphate (IMP) by 5-aminimidazole-4-carboxamide ribonucleotide transformylase/IMP cyclohydrolase (ATIC) in the last two steps of the de novo purine synthesis pathway (Figure 1). MTX slows this conversion by inhibiting ATIC (4, 10–12). Instead of being converted to IMP, ZMP can be dephosphorylated to form 5-aminimidazole-4-carboxamide-1-β-d-ribofuranoside (AICAR), which is then excreted in the urine (13, 14). Congenital ATIC deficiency markedly increases the concentration of ZMP in erythrocytes and in urinary excretion of AICAR (13), thus indicating that ATIC is essential for ZMP conversion to IMP. In RA (15) and PsA (16) patients, MTX increases excretion of ZMP.
of 4-aminomidazole-5-carboxamide (AICA), a urinary AICAR metabolite. Increased AICA excretion suggests that MTX inhibits ATIC and induces intracellular accumulation of ZMP in these patients. However, while ZMP accumulation was noted during MTX treatment in a mouse arthritis model (4), ZMP has not been directly measured in humans during chronic MTX therapy. Clearly, the link between ZMP accumulation and metabolic improvements in patients receiving MTX therapy needs to be evaluated.

In the current study we aimed to determine whether chronic ZMP accumulation underlies metabolic improvements in RA and PsA patients on MTX therapy. To evaluate whether ZMP accumulated during MTX therapy, we measured the concentration of ZMP in erythrocytes and the concentration of its dephosphorylated derivative AICAR in urine by using recently developed stable isotope dilution liquid chromatography mass spectrometry (LC-MS/MS) assays (14, 17). We found that concentrations of ZMP and AICAR were not increased during the first 6 months of MTX therapy. MTX reduced the concentration of glycated haemoglobin (HbA1c) and suppressed inflammation but these changes were not correlated with increased erythrocyte ZMP or urinary AICAR concentrations. Collectively, our data suggest that amelioration of glucose homeostasis and suppression of inflammation during MTX therapy are not associated with chronic accumulation of ZMP.

Method

This study was approved by the Ethics Committee of the Slovenian Ministry of Health and was conducted according to the principles outlined in the Declaration of Helsinki (version VI, originally adopted in 1964). All patients provided written informed consent before inclusion.

Patients

A 6-month prospective study was performed at the Department of Rheumatology, University Medical Centre Ljubljana. Twenty-eight newly diagnosed patients with RA or PsA (none of whom were professional athletes or known users of illicit drugs) fulfilled the inclusion criteria and were enrolled between December 2012 and June 2013. RA diagnosis was based on the American College of Rheumatology/European League against Rheumatism (ACR/EULAR) 2010 classification criteria. PsA diagnosis was based on Classificiation of Psoriatic ARthritis (CASPAR) criteria. Patients with diabetes mellitus or hypothyroidism were not enrolled. All patients were MTX naïve at inclusion.

Protocol

The assessment at inclusion and before the initiation of MTX therapy included the disease activity score in 28 joints based on erythrocyte sedimentation rate (DAS28-ESR), body mass index (BMI), routine blood samples for determination of fasting plasma glucose and insulin, homeostasis model assessment of insulin resistance [HOMA-IR: glucose (mmol/L) × insulin (µU/mL)/22.5], HbA1c, triglycerides, high density lipoproteins (HDL), low density lipoproteins (LDL), urate, urine creatinine, and additional blood and urine samples for the determination of ZMP and AICAR, respectively. After this initial assessment, patients were started on 15 mg of MTX weekly. Erythrocyte ZMP and urinary AICAR were re-evaluated after 1 and 6 months of treatment. All other parameters were determined at regular check-ups at 1, 2, 3, and 6 months. Blood and urine samples for biochemical analyses were collected on the day before the administration of the regular weekly MTX dose. Clinical assessment of all patients was carried out by the same physician throughout the study. MTX treatment with dose escalation in accordance with the treat-to-target approach was required. The maximal MTX dose was 25 mg/week (oral or subcutaneous application). Glucocorticoids were administered intramuscularly (120 mg of methylprednisolone) once a month or through an oral tapering

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scheme (4–8 mg methylprednisolone per day), if required. All patients received folic acid (10 mg/week) during MTX treatment.

**Routine blood biochemistry**

For the determination of glucose, C-reactive protein (CRP), total cholesterol, HDL, LDL, triglycerides, creatinine, and insulin, venous blood was collected in plain serum tubes without anticoagulant, while plasma tubes containing ethylenediaminetetraacetic acid (EDTA) were used for HbA1c and ESR. To obtain serum, blood samples were left at room temperature (RT) for 30 min and were subsequently centrifuged (1800 g, 10 min, RT). Serum was aliquoted into polypropylene tubes and labelled. Samples were kept at 4°C and analysed the same day. Alternatively, samples were stored at −20°C until further analysis. Blood samples for ESR estimation were stored at 4°C for no longer than 6 h. Glucose was measured using the hexokinase assay and CRP using a latex-enhanced immunoturbidimetric assay. Total cholesterol was measured using an enzymatic assay. LDL and HDL were measured using an elimination/catalase assay. A glycerol phosphate oxidase-based assay was used to measure triglycerides. Urate was measured using a uricase/peroxidase assay. Creatinine was measured using Jaffe’s (alkaline picrate) reaction. All measurements were performed with the ADVIA 1800 chemistry system (Siemens, Germany). Insulin was determined using a chemiluminescence immunoassay. For determination of HbA1c, centrifugation was performed at 4°C (1800 g, 10 min), the supernatant was aliquoted into polypropylene tubes, labelled, and stored at −20°C until analysis (capillary electrophoresis). ESR was determined by the Westergren method.

**Determination of erythrocyte ZMP and urine AICAR concentrations**

For the determination of erythrocyte ZMP and urinary AICAR concentrations, blood and urine samples were collected during the initial assessment and after 1 and 6 months of MTX therapy. Blood was drawn into a 2-mL lithium heparin tube and immediately filled with dipyridamole and A-13497 (both from Sigma-Aldrich, Buchs, Switzerland) to a final concentration of 25 μM and 25 nM, respectively, and stored on ice. To separate plasma from erythrocytes, blood samples were centrifuged for 10 min at 1300g (at 25°C). Erythrocytes were subsequently washed and centrifuged (1300g, 25°C) three times with phosphate-buffered saline (pH 7.4). Lysates were prepared by adding distilled water to centrifuged erythrocytes. The volume of added water was equal to the volume of plasma that had been initially removed. Erythrocyte lysates were stored in 1.5-mL aliquots at −80°C until analysis. Urine samples were immediately cooled (4°C), divided into 3-mL aliquots and then stored at −80°C until analysis. Quantification of erythrocyte ZMP and urinary AICAR was conducted elsewhere using isotope dilution LC-MS/MS approaches as described previously (10, 13). AICAR was normalized to urinary creatinine concentration. MTX had no effect on urinary creatinine concentration (data not shown). The concentration of ZMP in erythrocytes was calculated by dividing the ZMP concentration in erythrocyte lysates by the haematocrit.

**Statistical analyses**

Repeated-measures analysis of variance (ANOVA) was used for normally distributed measurements, while a Friedman test was used for non-normally distributed measurements. Post-hoc ANOVAs with Bonferroni correction were conducted to assess pairwise differences between measurements after 1, 2, 3, and 6 months of the MTX therapy and the measurements at the start of the therapy. Relationships between measurements were studied by applying linear regression analysis (see Figures 2 and 3). All statistical analyses were performed with SPSS, version 21.0 (IBM Corp, Armonk, NY, USA).

**Results**

**Participants**

Twenty-six (10 male, 16 female) patients with newly diagnosed RA (n = 16) or PsA (n = 10) were included in this study. Ten RA patients were positive for rheumatoid factor. Six of these were also positive for anticyclic citrullinated peptide (anti-CCP) antibodies. Two patients had only anti-CCP antibodies. The initial cohort included two more patients. However, in one case MTX had to be discontinued due to adverse effects and one patient had to be excluded due to non-compliance. While all 26 patients were DMARD naïve at baseline, three patients received glucocorticoids before inclusion in this study (Table 1). Patients did not receive biologicals or any other DMARD during the study. The mean age of the participants was 53 ± 12 (range 27–76) years.

**Disease activity during MTX treatment**

ESR, CRP, and DAS28-ESR were markedly reduced with treatment (Table 1), such that after 6 months 20 out of 26 patients were in remission or had low-activity disease (DAS28-ESR ≤ 3.2). The remaining six patients had moderately active disease (3.2 < DAS28-ESR ≤ 5.1) at 6 months.
Glucose homeostasis and lipid profile during MTX treatment

Fasting plasma glucose (Table 1) and HOMA-IR (Figure 2), an index of insulin resistance, remained unaltered during the 6 months of MTX treatment. Conversely, the concentration of HbA1c was reduced (Figure 2) in 20 out of 26 patients at 6 months. As assessed by the concentration of HbA1c, (5.7% ≤ HbA1c ≤ 6.4%), the number of pre-diabetic patients was reduced from 18 (at baseline) to 7 (at 6 months). Triglycerides, total cholesterol, and LDL did not increase during MTX therapy (Table 1). Conversely, HDL concentration increased steadily and reached its highest level at 6 months. We also observed a trend towards a reduction in the LDL:HDL ratio at 6 months of MTX therapy (p < 0.10).

Glucose homeostasis and lipid profile during MTX treatment

Erythrocyte ZMP and urinary AICAR concentrations during MTX treatment

ZMP and AICAR were detectable in erythrocytes and urine before the initiation of therapy with MTX (Figures 3A and 3B). Concentrations of ZMP and AICAR did not differ between RA and PsA patients (Supplementary Figure 1). The median concentration of ZMP in erythrocytes was not increased at 1 month or at 6 months of MTX therapy (Figure 3A). On the contrary, we observed a trend towards a reduction in erythrocyte ZMP concentration at 6 months (p < 0.10). Urinary AICAR concentration remained unaltered during the 6 months of therapy with MTX (Figure 3B). Erythrocyte ZMP and urinary AICAR concentrations also remained unaltered during MTX therapy if RA and PsA patients were analysed separately (Supplementary Figure 1).

MTX is known to inhibit ATIC, which converts ZMP to IMP, in a concentration-dependent manner (11, 12). The concentration of MTX in erythrocytes in RA patients depends on the weekly MTX dose (18). We therefore predicted that a higher MTX dosage might lead to more efficient ATIC inhibition and subsequently to accumulation of ZMP, at least in those patients who received the highest MTX doses. The cumulative 6-month dose of MTX was 518 ± 80 (range 335–665) mg. These cumulative doses of MTX were plotted against the differences in ZMP or AICAR concentrations between baseline and 6 months. We found that alterations in ZMP or AICAR concentration did not correlate with the cumulative dose of MTX (Figures 3C and 3D).

HbA1c concentration and DAS28-ESR in relation to erythrocyte ZMP and urinary AICAR concentrations

Overall, MTX therapy did not increase erythrocyte ZMP and urinary AICAR concentrations. Nevertheless, ZMP and AICAR were increased in 40–50% of patients. We therefore determined whether this increase correlated with the reduction in HbA1c or DAS28-ESR. HbA1c concentration at 6 months was reduced in patients with elevated as well as diminished erythrocyte ZMP concentration (Figure 4A and Supplementary Figure 2). Changes in HbA1c and ZMP concentrations from baseline to 6 months were not correlated. Conversely, the reduction in HbA1c concentration correlated with the decrease in urinary AICAR concentration (Figure 4B and Supplementary Figure 2). Reduction in DAS28-ESR at 1 month or 6 months did not correlate with changes in erythrocyte ZMP or urinary AICAR concentrations (Figure 4C and 4D).

Discussion

Intracellular accumulation of ZMP is thought to be one of the major mechanisms underlying MTX-induced
adenosine release and suppression of inflammation (4, 19). ZMP is also a direct AMPK activator (5), suggesting that accumulation of ZMP may also lead to metabolic improvements through AMPK activation (8, 9). ZMP metabolism during MTX therapy was traditionally assessed by measuring excretion of AIC, a urinary AICAR metabolite (15, 16, 20, 21). However, whether MTX therapy induces intracellular accumulation of ZMP in RA or PsA patients has not been proven by direct experimental evidence. In this study we assessed ZMP metabolism directly by measuring erythrocyte ZMP and urinary AICAR concentrations. We determined that ZMP and AICAR concentrations were not chronically increased during the first 6 months of MTX therapy. We also determined that improved glucose homeostasis and suppression of inflammation were not associated with chronic increase in ZMP or AICAR concentrations.

Our data show that MTX therapy does not increase erythrocyte ZMP and urinary AICAR concentrations. This was unexpected because MTX is a well-characterized ATIC inhibitor (10–12). Once inside the cell, MTX is converted to MTX polyglutamates, which are up to 25,000-fold more effective ATIC inhibitors than the native MTX (10). During MTX therapy, concentrations of MTX polyglutamates in erythrocytes progressively increase for several months before reaching a steady state (22). Inhibition of ATIC by MTX polyglutamates is concentration dependent (11, 12), thus the lowest ATIC activity was expected after 6 months of MTX therapy. Low ATIC activity is reported to increase erythrocyte ZMP and urinary AICAR concentrations (13). In our study MTX did not consistently increase the concentration of ZMP or AICAR, suggesting that ATIC inhibition was not sufficient to produce chronic accumulation of

Figure 3. Concentrations of 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranosyl 5’-monophosphate (ZMP) in erythrocytes and its dephosphorylated derivative 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) in urine. Newly diagnosed disease-modifying anti-rheumatic drug (DMARD)-naïve patients with rheumatoid arthritis (RA) or psoriatic arthritis (PsA) were treated with MTX for 6 months. (A) ZMP concentration in erythrocytes (n = 25–26) and (B) AICAR concentration in urine (n = 24–25) were determined using liquid chromatography mass spectrometry (LC-MS/MS) at baseline and after 1 month and 6 months of MTX therapy. Whiskers represent the 10th and 90th percentiles. AICAR concentration was normalized to the urinary concentration of creatinine. (C) Change in erythrocyte ZMP concentration between 6 months and baseline plotted against the cumulative dose of MTX at 6 months (n = 25). (D) Change in urinary AICAR concentration between 6 months and baseline plotted against the cumulative dose of MTX at 6 months (n = 24). Missing values: one ZMP and one AICAR sample had to be excluded as outliers (> 4 sd from the mean), while four urinary samples were unavailable for AICAR determination.
Table 1. Clinical and laboratory parameters during therapy with methotrexate (MTX) for 0, 1, 2, 3, and 6 months.

| Variable     | 0       | 1       | 2       | 3       | 6       |
|--------------|---------|---------|---------|---------|---------|
| DAS28-ESR    | 5.3 ± 1.3 | 4.1 ± 1.3*** | 3.5 ± 0.9*** | 3.0 ± 1.0*** | 2.8 ± 0.9*** |
| ESR (mm/h)   | 34.2 ± 25.0 | 27.3 ± 21.3 | 21.2 ± 14.4*** | 20.7 ± 17.3*** | 20.2 ± 14.1*** |
| CRP (mg/L)†  | 12.5 (< 5.0–32.8) | < 5.0 (< 5.0–10.0)** | < 5.0 (< 5.0–8.0)** | < 5.0 (< 5.0–7.3)** | < 5.0 (< 5.0–8.0)** |
| BMI (kg/m²)  | 28.5 ± 4.6 | 28.2 ± 4.5 | 28.2 ± 4.6 | 28.1 ± 4.5 | 28.4 ± 4.5 |
| Glucose (mmol/L) | 5.4 ± 0.5 | 5.4 ± 0.5 | 5.3 ± 0.5 | 5.4 ± 0.5 | 5.5 ± 0.5 |
| Total cholesterol (mmol/L) | 5.3 ± 1.3 | 5.4 ± 1.3 | 5.7 ± 1.3 | 5.7 ± 1.2 | 5.6 ± 1.1 |
| LDL (mmol/L) | 3.3 ± 1.1 | 3.4 ± 1.0 | 3.7 ± 1.0 | 3.5 ± 1.0 | 3.4 ± 0.9 |
| HDL (mmol/L) | 1.4 ± 0.4 | 1.5 ± 0.5 | 1.5 ± 0.4** | 1.5 ± 0.4** | 1.6 ± 0.4** |
| LDL/HDL ratio | 2.6 ± 0.9 | 2.5 ± 0.9 | 2.5 ± 0.8 | 2.4 ± 0.8 | 2.3 ± 0.7 |
| Triglycerides (mmol/L) | 1.4 ± 0.7 | 1.2 ± 0.6 | 1.2 ± 0.5 | 1.4 ± 0.7 | 1.5 ± 0.7 |
| Creatine (μmol/L) | 300 ± 77 | 308 ± 76 | 298 ± 69 | 308 ± 70ª | 307 ± 68 |
| MTX (mg/week) | 60.3 ± 13.1 | 63.5 ± 14.2 | 62.7 ± 13.4 | 63.6 ± 11.7 | 64.2 ± 13.8 |
| Methylprednisolone | 0 | 15 (15–20) | 15 (10–20) | 20 (10–25) | 20 (10–25) |
| 4–8 mg po (patients) | | | | | |
| DAS28-ESR, Disease activity score in 28 joints based on erythrocyte sedimentation rate; CRP, C-reactive protein; BMI, body mass index; LDL, low density lipoprotein; HDL, high density lipoprotein.

Results are expressed as mean ± standard deviation, median (interquartile range), or number of patients, as appropriate.

At 2 months n = 25, for all others n = 26 or as indicated (ªn = 25, ªn = 24). Missing values: at 2 months one patient failed to attend the check-up, other missing values are due to unavailable samples.

*p < 0.05, **p < 0.01, ***p < 0.001 post-hoc analysis significance levels of pairwise differences between current measurement and measurement at the start of MTX therapy (measurement at 0). Methylprednisolone was administered 120 mg intramuscularly (im) once a month or 4–8 mg orally (po) once a day.

† The lower limit of detection was 5 mg/L.

ZMP. Consistent with this view, urinary excretion of AICA was increased in RA patients on the day of MTX administration, but not before the regular weekly MTX dose (16). Thus, while acute administration of MTX may transiently increase excretion of AICA (15, 16), our results indicate that MTX therapy does not lead to chronic accumulation of ZMP.

Erythrocytes cannot synthesize ZMP in the de novo purine synthesis pathway, but they can form ZMP from AIC or by phosphorylating AICAR (17, 23, 24). This raises the question of whether concentration of ZMP in erythrocytes reflects ZMP and AICAR turnover in tissues that are capable of synthesizing purines de novo. However, despite differences in purine metabolism, erythrocyte ZMP concentration is markedly increased in congenital ATIC deficiency (13). This demonstrates that, given sufficient reduction of ATIC activity, slowed ZMP clearance in other tissues is paralleled by an increased concentration of ZMP in erythrocytes. Furthermore, erythrocyte ZMP concentrations probably mirror long-term plasma concentrations of AICAR due to slow dephosphorylation of ZMP and the long lifetime of erythrocytes (17). Finally, erythrocytes, which are capable of converting ZMP to IMP through ATIC (23), accumulate MTX polyglutamates during treatment of RA (22). This indicates that ZMP metabolism in erythrocytes is directly targeted by MTX. Thus, although the tissue-specific variations in MTX action cannot be excluded, erythrocyte ZMP concentration may still be a valid marker for the assessment of tissue ZMP metabolism during MTX therapy.

MTX therapy did not increase erythrocyte ZMP concentration or AICAR excretion, indicating that activity of ATIC was not suppressed. Erythrocyte MTX polyglutamates, which inhibit ATIC much more efficiently than the parent drug (10), have elimination half-lives of 2–4 weeks and become undetectable only 4–10 weeks after termination of MTX therapy (22). Suppression of ATIC was therefore expected, although we measured ZMP and AICAR 6 days after administration of MTX. One reason for the failure to observe ZMP accumulation could have been the folic acid supplementation that the RA and PsA patients received throughout the study to reduce the risk of adverse effects. Indeed, the folic acid derivative 10-formyltetrahydrofolate, which is required for ATIC-catalysed conversion of ZMP to IMP, can markedly reduce the inhibitory effects of MTX on ATIC (10). Another possibility of interest is that inhibition of other MTX targets obscured suppression of ATIC. For instance, MTX also inhibits glycine-namide ribonucleotide transformylase (GART) (10), an enzyme upstream from ATIC in the de novo purine synthesis pathway (Figure 1). Inhibition of GART would slow the flux through this pathway and thereby reduce the synthesis of ZMP (25). Of note, we observed a trend towards reduction of erythrocyte ZMP concentration at 6 months, which may reflect suppression of purine synthesis in tissues with an active de novo pathway.

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We have shown that 6 months of MTX therapy reduces the concentration of HbA1c in RA and PsA patients. A similar result was recently obtained in RA patients after 3 months of therapy with MTX (2). A reduced concentration of HbA1c indicates that MTX therapy improves long-term glucose homeostasis, consistent with the epidemiological data that suggest that MTX could reduce the risk of diabetes mellitus and the metabolic syndrome in RA and PsA (1, 3). MTX also alleviates hyperglycaemia in diabetic mice (9), which again supports the notion that MTX protects against metabolic complications associated with RA and PsA patients. We hypothesized that these metabolic effects might be associated with MTX-induced accumulation of ZMP, which would tend to activate AMPK (6, 8). However, the decrease in HbA1c concentration was not correlated with increased erythrocyte ZMP concentration or urinary AICAR excretion, indicating that sustained MTX-induced suppression of ATIC is not required for this effect. Consistent with this view, experiments in isolated skeletal muscle show that ATIC inhibition does not provide the exclusive mechanism through which MTX promotes glucose uptake and AMPK activation (8). Although the underlying mechanism remains unclear, our results support the notion that MTX alleviates glucose homeostasis in RA and PsA patients.

Our study indicates that MTX therapy does not increase erythrocyte ZMP and urinary AICAR concentrations. Molecular mechanisms underlying the pharmacological effects of MTX in RA and PsA patients are likely to be similar. Indeed, clinical and laboratory responses to the initiation of MTX therapy were similar between RA and PsA patients in our cohort. However, our cohort was relatively small and heterogeneous and we cannot exclude the possibility that the effects of MTX

Figure 4. Glycated haemoglobin (HbA1c) concentration and disease activity score in 28 joints based on erythrocyte sedimentation rate (DAS28-ESR) in relation to concentrations of 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranosyl 5′-monophosphate (ZMP) in erythrocytes and its dephosphorylated derivative 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) in urine. Concentrations of HbA1c (%) were measured to evaluate glucose homeostasis in patients with rheumatoid arthritis (RA) or psoriatic arthritis (PsA) during 6 months of MTX therapy. Inflammatory activity was estimated by DAS28-ESR. Change in concentrations of HbA1c (%) plotted against change in (A) erythrocyte ZMP concentration (n = 25) or (B) urinary AICAR concentration (n = 24) between baseline and 6 months. Change in DAS28-ESR plotted against change in (C) erythrocyte ZMP concentration (n = 25–26) or (D) urinary AICAR concentration (n = 24) from baseline to 1 month (white squares) or from baseline to 6 months (black circles). Missing values: one ZMP and one AICAR sample had to be excluded as outliers (> 4 sd from the mean), while four urinary samples were unavailable for AICAR determination.
on ZMP metabolism might be different between RA and PsA patients. Thus, disease-specific factors might have obscured the MTX-induced accumulation of ZMP or increased urinary AICAR excretion. In addition, variability in MTX pharmacokinetics might have affected the results. For instance, MTX dosage is not the only factor that determines the concentration of MTX polyglutamates in erythrocytes (18). Thus, even patients who received a similar cumulative dose of MTX might have had different intracellular concentrations of MTX polyglutamates, which would tend to affect the level of ATIC inhibition and therefore the concentration of ZMP in erythrocytes. Despite these reservations, HbA1c and DAS28-ESR were reduced in patients with increased or decreased ZMP or AICAR concentrations, indicating that accumulation of ZMP is not an exclusive mechanism linking MTX to improvements in metabolic status or suppression of inflammation.

In conclusion, our data indicate that MTX therapy does not produce a chronic increase in erythrocyte ZMP concentration and urinary AICAR excretion in patients with RA and PsA. Our data also indicate that neither the reduction in HbA1c concentration nor the reduction in DAS28-ESR during MTX therapy is correlated with increased erythrocyte ZMP or urinary AICAR concentrations. Collectively, our study suggests that amelioration of glucose homeostasis and suppression of inflammation during MTX therapy are not associated with chronic accumulation of ZMP.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Supplementary Figure 1. Effects of MTX therapy were similar in RA and PsA patients.

Supplementary Figure 2. MTX therapy reduced HbA1c concentration in patients with increased and decreased erythrocyte ZMP and urinary AICAR concentrations.

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