TREXATE SUPPORT

Folate deficiencies caused by chronic use of low-dose methotrexate

Companion Therapeutics

Scientific overview

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TABLE OF CONTENTS

1. DESCRIPTION AND USE

TREXATE Support is a dietary supplement specifically designed to be administered once a day, five days a week, in order to maintain healthy levels of vitamins and amino acids which may be significantly reduced in patients who consume methotrexate on a long-term basis.

Folate deficiency is the main mechanism by which the patients treat with methotrexate, develop gastrointestinal, mucocutaneous, hematological and hepatic adverse effects.

The product could also be useful in patients with long treatments of other dihydrofolate reductase inhibitors (pemetrexed, trimethoprim or pyrimethamine) which according to medical criteria, are in high risk of suffering folate deficiency.

2. PHARMACOLOGICAL BACKGROUND

Methotrexate (MTX) is a folic acid antagonist, widely used for the treatment of inflammatory disorders for more than 50 years. (Prey & Paul, 2009) MTX inhibits dihydrofolate reductase (DHFR), resulting in a decreased supply of folates. At high doses, MTX inhibits purine and pyrimidine synthesis, rendering it useful for many malignancies. At low doses (< 20 mg/week), MTX is commonly used as a disease modifying antirheumatic drug (DMARD) for treating rheumatoid arthritis (RA) and juvenile rheumatoid arthritis (JRA), as well as a first line agent for systemic therapy of severe psoriasis. (Shea, et al., 2013)

Adverse effects of MTX are mainly related to folate antagonism and/or folate deficiency. Folate supplementation, in the form of folic acid or folinic acid, is often coadministered with MTX to minimize adverse effects. (Prey & Paul, 2009)

During the treatment of malignancies, when high doses of MTX are used, calcium folinate injections are often included into the treatment protocol. However, when low doses are used, such as in the treatment of psoriasis, rheumatoid arthritis and juvenile rheumatoid arthritis, folate supplementation by oral route is not commonly used, even when it may be justified.

Pemetrexed causes the same folate deficiency, however this drug is only approved for use in malignancies at high doses, so the oral supplements of folates are less frequent required. (Cancer network, 2001) (Nakagawa, et al., 2006) (Yang, et al., 2013) (Takagi, et al., 2014)

Although less frequently, other drugs with a similar mechanism of action have been reported to cause the same adverse effects; for example, pyrimethamine, widely used in malaria, and trimethoprim. (Nzila, Okombo, & Molloy, 2013)

In addition to folate deficiency MTX is highly hepatotoxic, so it may induce a variety of histologic changes in the liver, including steatosis, stellate (Ito) cell hypertrophy, anisonucleosis (nuclei of varying sizes), and hepatic fibrosis. (Kremer, 2016)

3. NUTRITIONAL DEFICIENCY MECHANISM

Folic acid, also known as vitamin B9, cannot be synthetized by the body, so it must be acquired from the diet. Before exerting its biological function, folic acid needs to be reduced to its active form, the tetrahydrofolate (THF). This reduction process is a two-step reaction; first, the folic acid must be converted into dihydrofolate (DHF). Afterwards, DHF must be converted into THF. These two steps are catalyzed by the enzyme DHFR.

Once folate ion reaches its active form, it is ready to be used by the body in a series of important processes such as the synthesis of purines and pyrimidines, which in turn build the DNA and RNA, as well as the methylation of DNA, RNA, proteins and lipids.

MTX inhibits the enzyme DHFR, thereby obstructing folic acid activation, and blocking the biological processes in which it participates.

This blockage in the DNA and RNA synthesis is precisely how MTX exerts its therapeutic effect as an oncologic drug; but, at the same time, this is the mechanism by which it produces its well-known adverse effects. (See figure 1)

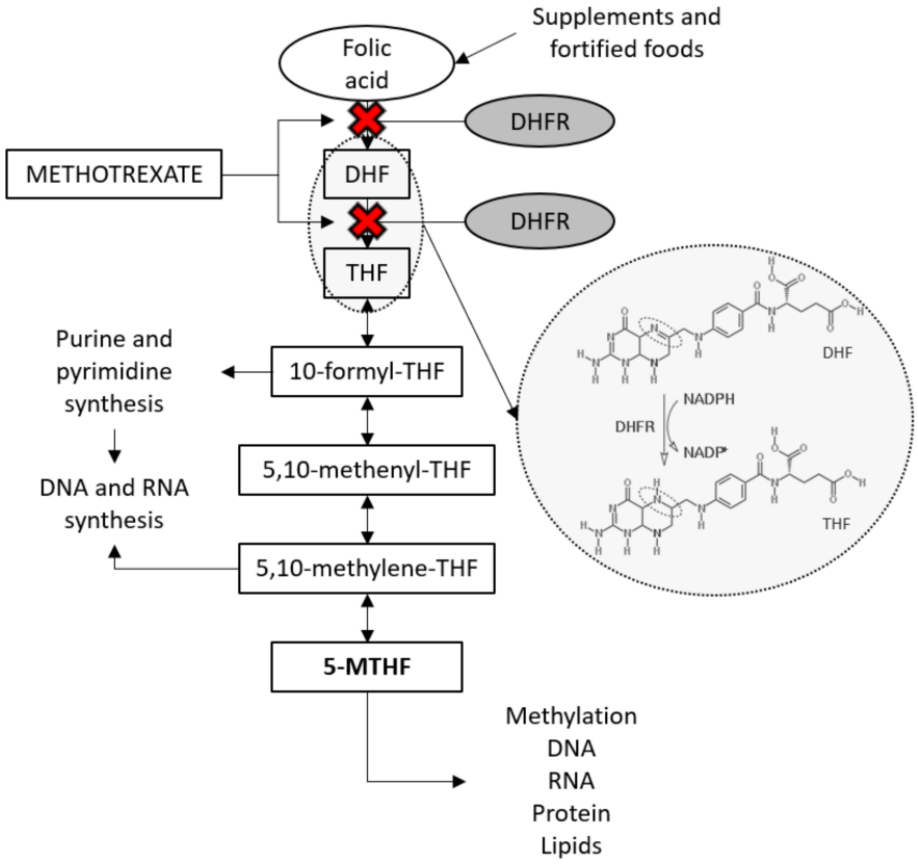

Figure 1. Folic acid activation pathway

According to that, chronic use of low dose MTX causes THF deficiency due to exactly the same mechanism by which the drug exerts its pharmacological action, the inhibition of the DHFR.

In addition to the synthesis of purines, pyrimidines, THF plays an important role in the metabolism of methionine, serine and glycine, as shown in the figure 2.

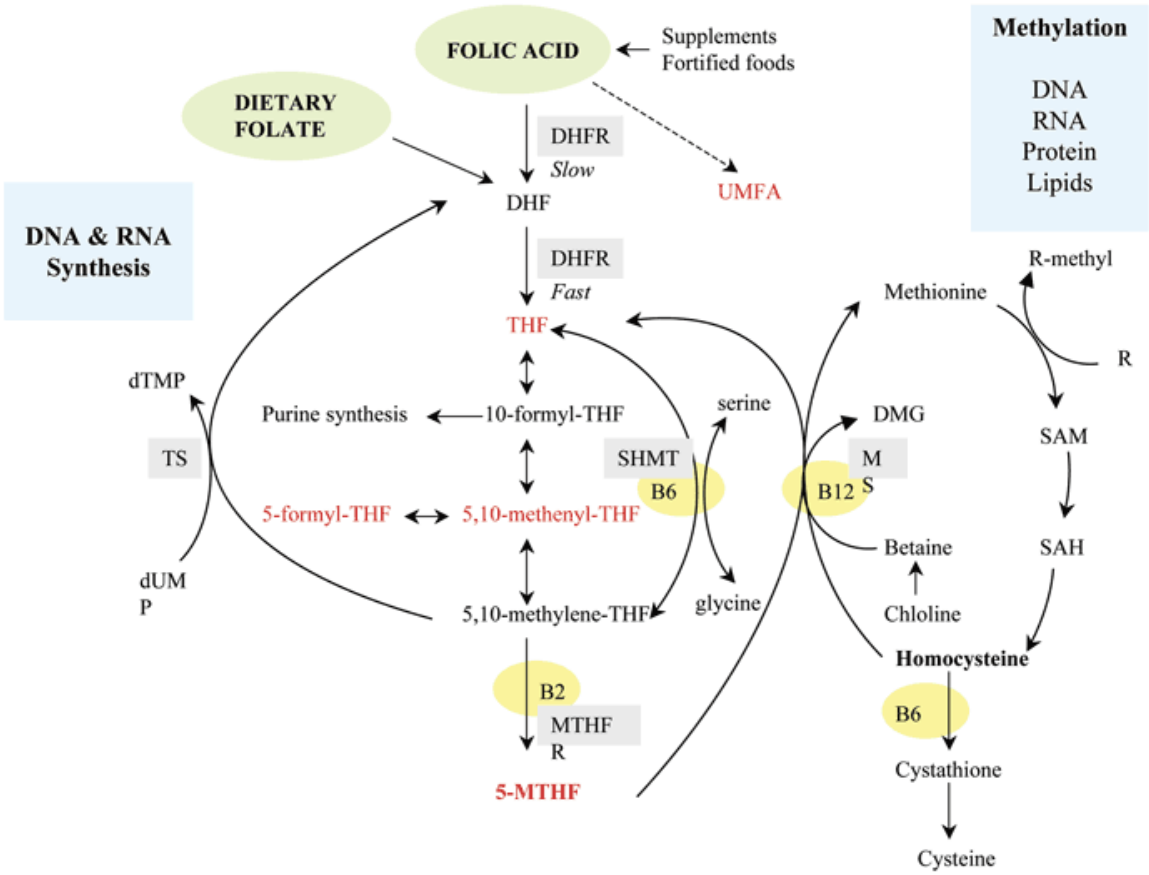

Figure 2. Folic acid metabolic pathway

Figure 2 also shows other cofactors involved in folic acid metabolic pathway such as vitamins B2, B6 and B12.

4. RELATED ADVERSE EFFECTS

The adverse effects related to folate deficiency can be classified in 4 groups: gastrointestinal, mucocutaneous, hematological and hepatic adverse effects.

4.1. Gastrointestinal (Nausea, abdominal pain and vomiting)

Gastrointestinal adverse effects are common after low-dose MTX treatment, affecting as many as 66% of patients. Due to these adverse effects, up to 12% of RA and Psoriatic arthritis (PsA) patients discontinue MTX after 6 months to 2 years of treatment. (Bulatović Ćalasan, et al., 2013)

Bulatović Cálasan and colleagues, from the University Medical Center, Wilhelmina Children's Hospital in Utrecht, (Bulatović Ćalasan, et al., 2013) used the MTX Intolerance Severity Score (MISS) -- previously validated in juvenile idiopathic arthritis patients -- to determine the prevalence of MTX intolerance in a cross-sectional study of 291 RA/PsA patients. The patients had low-to-moderate DAS-28 and were treated with MTX for at least three months.

The researchers defined MTX intolerance as having an MISS of at least 6 points, including at least one anticipatory, associative, or behavioral symptom.

Gastrointestinal symptoms emerged during MTX treatment in 123 (42.3%) of the patients, with nausea affecting 32.0%, abdominal pain 11.3% and vomiting 6.5%, the team reports.

Nearly one in 10 patients (8.6%) developed nausea in anticipation of treatment, and 11.0% had nausea even when thinking about the drug.

Behavioral symptoms were common, with 13.1% reporting restlessness, 10.0% reporting irritability, and 4.5% refusing to take the medication.

Overall, 32 patients (11.0%) met criteria for MTX intolerance (median, 9 points), including 10.4% of RA patients and 14.3% of PsA patients.

The most common symptoms in the patients with MTX intolerance were anticipatory (56.3%) and associative (53.1%) nausea, followed by anticipatory (37.5%) and associative (34.4%) abdominal pain. One in six intolerant patients (15.6%) even had anticipatory vomiting, compared with none of the tolerant patients.

Most of these symptoms represent a classical conditioning response to physical symptoms after MTX, the authors say.

MTX intolerance was significantly higher in patients receiving parenteral (20.6%) than in patients receiving oral (6.2%) MTX.

Using the same tool, the MISS questionnaire, Fatimah et al (Fatimah, et al., 2016) found similar results in RA patients with MTX. A total of 33.3 % of the subjects exhibited MTX intolerance. Out of which, the most recurring symptom of all was behavioral with a value of 44 % whereas vomiting was least noticeable with a figure of 11 %. The frequency of intolerance decreased with a decrease in weekly dose to a minimum of 20 % with 7.5 mg of MTX. (Fatimah, et al., 2016)

On the other hand, in a cross-sectional study including 179 JIA patients on MTX, van Dijkhuizen et al (van Dijkhuizen, et al., 2016) found that that the prevalence of MTX gastrointestinal intolerance in patients receiving subcutaneous MTX was at least as high as in patients receiving oral MTX, confirming that MTX gastrointestinal intolerance is not exclusive or oral treatments.

4.2. Mucocutaneous (Mouth and throat sores, mucositis, stomatitis and others)

Oral and intestinal epithelial cells are sensitive to the effects of MTX and may be frequently affected by mucositis. Erythematous, erosive and ulcerative oral lesions may be a consequence of MTX therapy. (Silva Pedrazas, Leitão de Azevedo, & Torres, 2010)

Figures 3 and 4 show some examples of mucocutaneous lesions caused by the prolonged use of low-dose MTX. (Troeltzsch, et al., 2013)

Figure 3. A) Erythematous and ulcerative buccal mucosa. B) Dry lips with concomitant cheilitis. C) Rashes and ulcers of the uvula and pharynx.

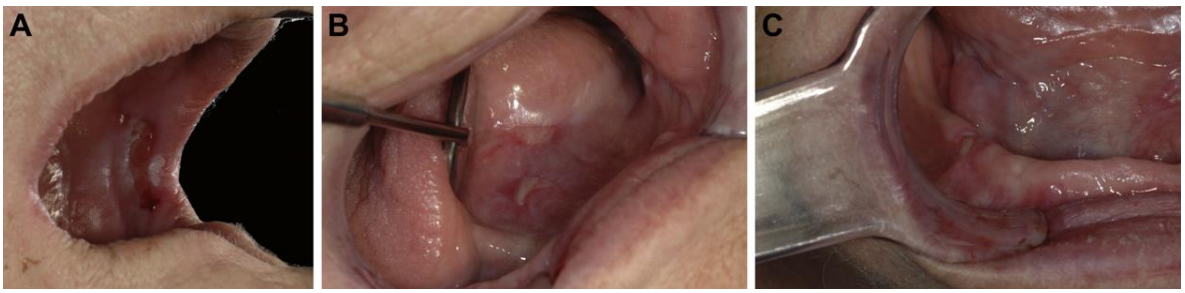

Figure 4. A) Ulcerative lesion on the buccal side of the corner of the mouth. B) Multiple lichenoid lesions on the floor of the mouth. C) Ulcer on the alveolar crest.

In a cross-sectional study, Silva Pedrazas et al (Silva Pedrazas, Leitão de Azevedo, & Torres, 2010) compared the frequency of oral complications in 28 RA patients treated with low-dose MTX vs 21 RA control patients undergoing regimens involving other therapies. Oral lesions were found in 22 patients (78.6%) undergoing MTX therapy, and in 5 patients (23.8%) undergoing other therapies ($p < 0.001$). According to that, the prevalence of oral mucosa lesions in RA patients receiving low doses of MTX therapy is higher than in RA patients not receiving the drug.

4.3. Hematological

When used in chemotherapy MTX causes profound suppression of bone marrow. However, even at a low dose it may be associated with bone marrow suppression—particularly in the presence of renal insufficiency or when other drugs are taken concomitantly. (Sosin & Handa, 2003)

4.4. Hepatic

MTX can induce a variety of histologic changes including steatosis, stellate (Ito) cell hypertrophy, anisonucleosis (nuclei of varying sizes), and hepatic fibrosis. (See figure 5) (Kremer, 2016) The mechanism by which MTX adversely affects the liver is unclear. Hepatic folate stores are depleted by MTX in the doses used in RA, and this folate depletion seems to be related with the hepatic toxicity, since supplementation with either folic acid 1 mg per day or folinic acid 2.5 mg per week is associated with a reduced incidence of serum transaminase elevation. (Prey & Paul, 2009) (Shea, et al., 2013)

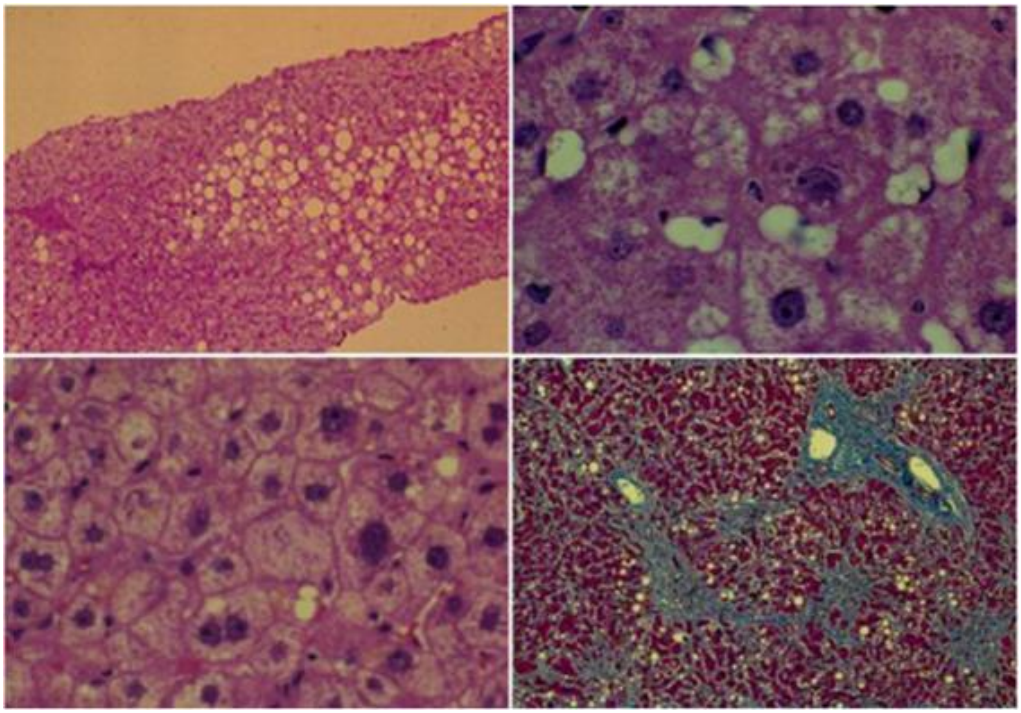

Figure 5. Spectrum of histological changes in methotrexate hepatotoxicity includes steatosis (top left), stellate (Ito) cell hypertrophy (top right), anisonucleosis (nuclei of varying sezes, bottom left), and, on Masson stain, hepatic fibrosis (bottom right)

Most of our understanding of the hepatotoxic potential of MTX comes from its use in nonmalignant disease, such as psoriasis and RA. MTX hepatotoxicity in patients with psoriasis appears to increase with the total cumulative dose. Cirrhosis and fibrosis occur more than twice as frequently in patients receiving daily MTX therapy when compared with those receiving intermittent dosing. (Kremer, 2016)

5. FORMULA

SUPPLEMENT	AMOUNT PER SERVING	% DAILY VALUE	RATIONALE
Folate (as 5-methyltetrahydrofolate)	1000,0 µg	250 %	Folate deficiency
Vitamin B12 (as methylcobalamin)	50,0 µg	833 %	Component of folate pathway
Vitamin B6 (as pyridoxine hydrochloride)	10,0 mg	500 %	Component of folate pathway
Vitamin B2 (as riboflavin)	5,0 mg	294 %	Component of folate pathway
Proprietary blend	400 mg	*	
Milk thistle extract (Silybum marianum) (standardized to 80% silymarin)			Hepatotoxicity
L-methionine			Component of folate pathway
L-serine			Component of folate pathway
L-glycine			Component of folate pathway

*** Daily value not stablished**

Serving size: 1 capsule
Servings per container: 30 servings or 30 capsules

6. FARMACEUTICAL DOSAGE FORM AND PACKAGING MATERIAL

"00" standard two-piece gelatin orange/orange capsule, where each capsule contains 1000 µg of 5-methyltetrahydrofolate + 50 µg of vitamin B12 + 10 mg of vitamin B6 + 5 mg of vitamin B2 + 400 mg of a proprietary blend containing milk thistle extract + L-methionine + L-serine + L-glycine.

30 capsules bottled in a 100 CC white HDPE bottle / 38mm white ribbed CRC cap with heat seal /4 color process label /cotton / desiccant.

7. RATIONALE OF THE COMPONENTS

7.1. Folate (as 5-methyltetrahydrofolate)

The efficacy of supplements containing folic and folinic acid, in the reduction of side effects associated to chronic consumption of DHFR inhibitors, has been widely proved through a series of clinical trials. (Prey & Paul, 2009) (Shea, et al., 2013)

In 2008, a systematic review was performed by Prey et al. (Prey & Paul, 2009) Cochrane and MEDLINE databases were systematically searched. Randomized controlled trials in patients treated with MTX for rheumatoid arthritis or psoriasis were included. Double-blind randomized placebo-

controlled trials were selected; and the analysis for each subgroup of side-effects was performed: (gastrointestinal, mucocutaneous, hematological and hepatic). Six randomized controlled trials met the inclusion criteria, (Salim, Tan, Ilchyshyn, & Berth-Jones, 2006) (van Ede, et al., 2001) (Griffith, et al., 2000) (Weinblatt, Maier, & Coblyn, 1993) (Shiroky, et al., 1993) (Morgan, et al., 1990) with a total sample of 648 patients. There were 257 patients in the placebo group, 198 patients treated with folic acid, and 193 patients treated with folinic acid. The statistical analysis showed a significant reduction of 35.8% of hepatic side-effects induced by MTX for patients with supplementation with folic or folinic acid (95% confidence interval -0,467 to -0,248), as shown in the figure 6:

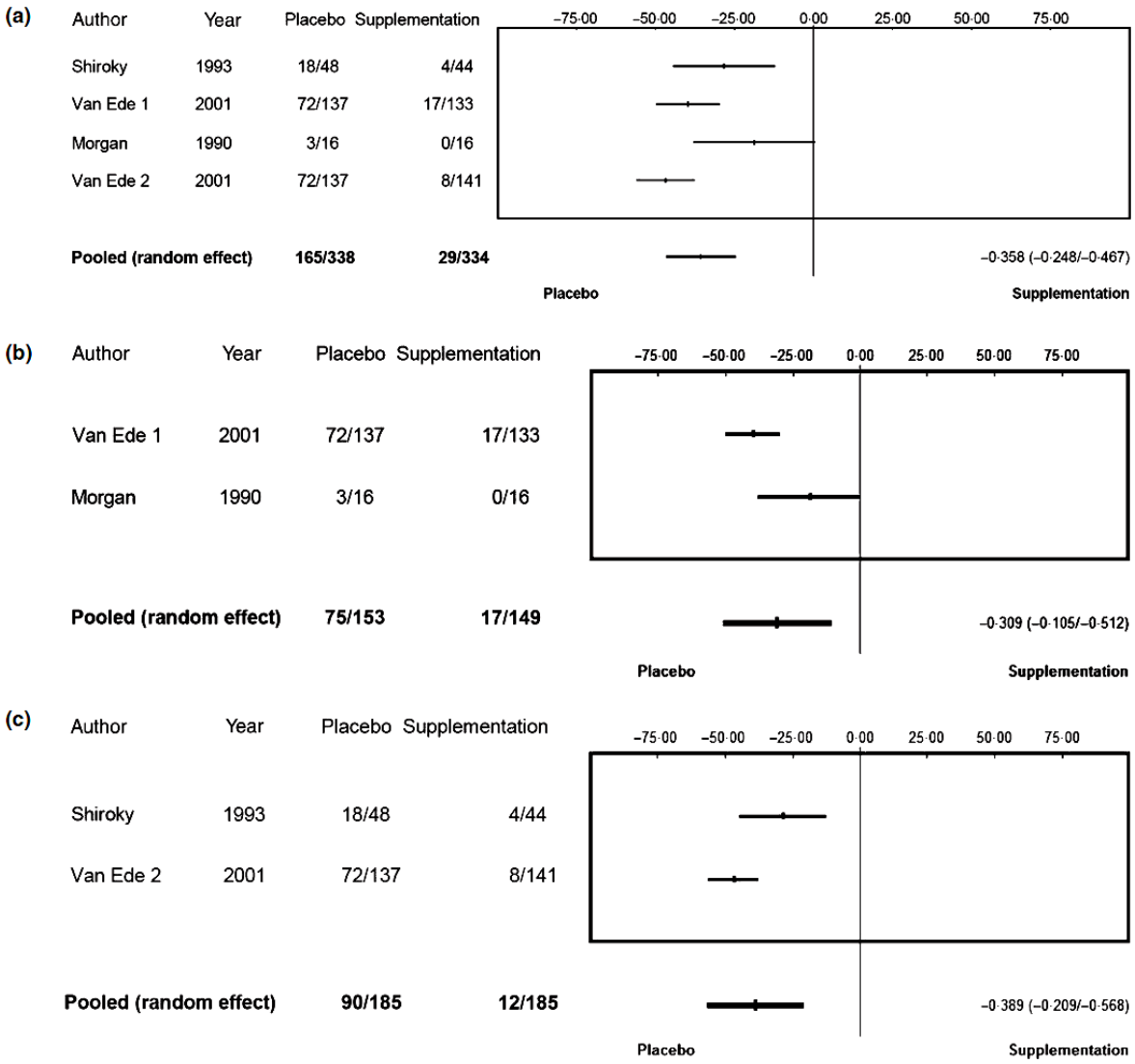

Figure 6. (a) Comparison of all supplementation vs. placebo, outcome hepatic side-effects. (b) Comparison of supplementation with folic acid vs. placebo, outcome hepatic side-effects. (c) Comparison of supplementation with folinic acid vs. placebo, outcome hepatic side-effects.

There was no statistical difference for mucocutaneous and gastrointestinal side-effects, although there was a trend in favor of supplementation as shown in the figures 7 and 8.

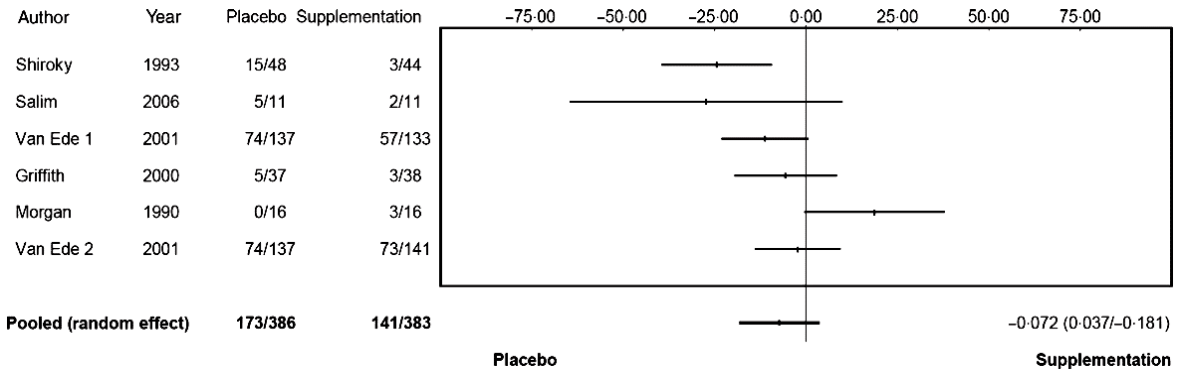

Figure 7. Comparison of supplementation vs. placebo, outcome mucocutaneous side-effects.

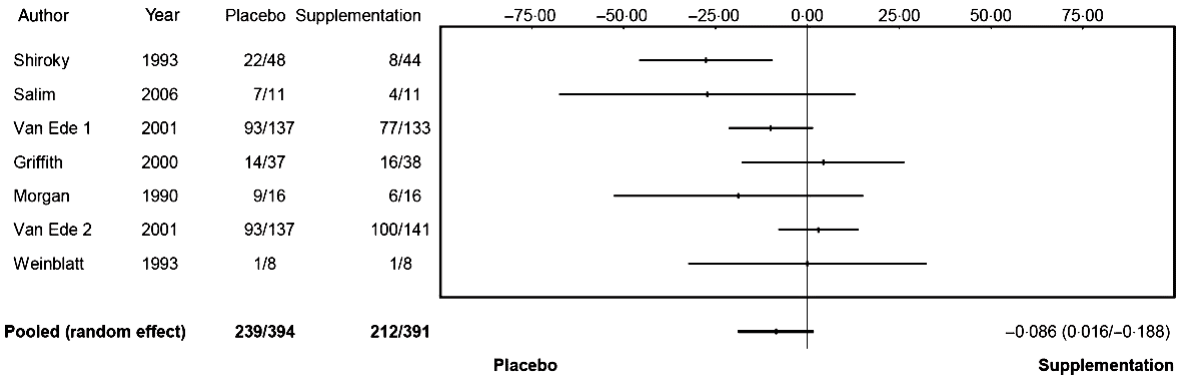

Figure 8. Comparison of supplementation vs. placebo, outcome gastrointestinal side-effects.

The effect of supplementation on hematological side-effects could not be assessed accurately due to a low incidence of these events in the population studied.

The results allow them to conclude that supplementation with folic acid is an effective measure to reduce hepatic adverse effects associated with MTX treatment. (Prey & Paul, 2009)

Six additional publications were excluded from the systematic review performed by Prey et al. (Prey & Paul, 2009) because of the breach of the inclusion criteria of this specific review. However, these six publications contain valuable information regarding the efficacy of folate supplementation in the reduction of adverse effects associated with low-dose MTX. Among the most significant there are two reviews:

The first is a meta-analysis performed by Ortiz et al. (Ortiz, Shea, Moher, Wells, & Tugwell, 1998) In this work, reviewed double blind randomized controlled trials (RCT) in which adult patients with RA were treated with low doses of MTX (< 20 mg/week), concurrently with folic or folinic acid. Of 11

trials retrieved, 7 met inclusion criteria. The total sample included 307 patients, of which 147 were treated with folate supplementation, 67 patients with folic, and 80 with folinic acid. A 79% reduction in mucocutaneous and gastrointestinal side effects was observed for folic acid [OR = 0.21 (95% CI 0.10 to 0.44)]. For folinic acid, a clinically but non-statistically significant reduction of 42% was found [OR = 0.58 (95% CI 0.29 to 1.16)]. Hematologic side effects could not be analyzed, since details by patients of each event were not provided. Their results support the protective effect of folate supplementation in reducing MTX side effects related to the mucocutaneous and gastrointestinal systems.

The second is a systematic review performed by Strober et al. (Ortiz, Shea, Moher, Wells, & Tugwell, 1998) According to this review, the use of folates increases the likelihood of efficacious long-term, tolerable, and toxicity-free therapy for patients receiving MTX. Again, the most significant benefits were related to mucocutaneous and gastrointestinal side effects.

The most recent and most relevant review was a Cochrane Collaboration® performed by Shea et al. (Shea, et al., 2013) In this work, the authors selected all double-blind, randomized, placebo-controlled clinical trials (RCTs) in which adult patients with rheumatoid arthritis were treated with MTX (at a dose equal to or less than 25 mg/week) concurrently with low-dose folate supplementation (a starting dose of < 7 mg weekly). Six trials with 624 patients were eligible for inclusion. The quality of the evidence was rated as 'moderate' for each outcome as assessed by GRADE, with the exception of hematologic side effects which were rated as 'low'. For patients supplemented with any form of exogenous folate (either folic or folinic acid) whilst on MTX therapy for rheumatoid arthritis, a 26% relative (9% absolute) risk reduction was seen for the incidence of GI side effects such as nausea, vomiting or abdominal pain (RR 0.74, 95%CI 0.59 to 0.92; P = 0.008). Folic and folinic acid also appear to be protective against abnormal serum transaminase elevation caused by MTX, with a 76.9% relative (16% absolute) risk reduction (RR 0.23, 95% CI 0.15 to 0.34; P < 0.00001), as well as reducing patient withdrawal from MTX for any reason (60.8% relative (15.2% absolute) risk reduction, RR 0.39, 95% CI 0.28 to 0.53; P < 0.00001). Additionally, the authors analyzed the effect of folic or folinic acid on the incidence of stomatitis /mouth sores, showing a trend towards reduction in risk.

According to the findings in the Cochrane review, the authors concluded that their results support a protective effect of supplementation with either folic or folinic acid for patients with rheumatoid arthritis during treatment with MTX. There was a clinically important significant reduction shown in the incidence of GI side effects, hepatic dysfunction (as measured by elevated serum transaminase levels) as well as a clinically important significant reduction in discontinuation of MTX treatment for any reason. A trend towards a reduction in stomatitis was demonstrated however this did not reach statistical significance.

7.1.1. Rationale of the administration interval and amount per serving

Since many of the therapeutic effects of MTX are related with the blockage of the DNA and RNA synthesis, which in turns in caused by the interference with DHFR, the obvious question is whether folate supplementation not only diminishes MTX's toxicity but also its efficacy.

Multiple studies have addressed this issue; most of them concluding the non-interference of folate supplementation in MTX efficacy, especially at low doses and when the administration of the folate supplement is separated from the MTX administration, for at least 24 hours.

Regarding rheumatoid arthritis, in 2014 after a systematic review of more than 50 publications, Cipriani et al (Cipriani, Ruscitti, Carubbi, Liakouli, & Giacomelli, 2014) concluded that low-dose folinic acid may decrease MTX toxicity, but high doses or early administration may diminish the therapeutic effect of MTX. Dosing between 12 and 48 hours after MTX administration might be preferable, as the available data suggest that dosing at this time frame offers the greatest promise.

During MTX treatment, at least 5 mg/week of folic acid is strongly recommended. (Shea, et al., 2013) (Cipriani, Ruscitti, Carubbi, Liakouli, & Giacomelli, 2014) (Visser, et al., 2009) Furthermore, only folinic acid at >5 mg/week was associated with a significant increase in the number of tender and swollen joints, whereas folic acid or low dosages (5 mg/week) of folinic acid were not. (Cipriani, Ruscitti, Carubbi, Liakouli, & Giacomelli, 2014) Therefore, evidence-based recommendations suggest folic acid supplementation of at least 5 mg/week, taking into account the potential need for higher dosages, with the currently higher doses of MTX. (Visser, et al., 2009)

The Italian consensus on the recommendations about the use of MTX for the treatment of rheumatic diseases with a focus on rheumatoid arthritis (De Leonardis, et al., 2010) recommend the supplementation of folic or folinic acid in patient with MTX in the following terms: "***In order to minimize side effects by MTX, without interfering with its effectiveness, folic acid or folinic acid may be administered. Folic acid can be administered either at a dosage of 10 mg / week, 24 hours after MTX or of 1 mg / day in the countries in which such presentation is available. The folinic acid must be administered at doses of ≤5 mg / week, 24 hours after MTX."***

other authors agree to recommend the prescription of at least 5 mg of folic acid week, under the argument that "folic acid supplementation significantly reduced liver and gastrointestinal toxicity without affecting efficacy". (Hernandez-Baldizon, 2012)

The beneficial effect of separating the administration of MTX and folic or folinic acid could be in part explained by the fact that folic acid and MTX are structurally similar and are partially absorbed by common membrane transporters; therefore, coadministration may affect their absorption. (Cipriani, Ruscitti, Carubbi, Liakouli, & Giacomelli, 2014)

On the other hand, compared with RA, there is a scarcity of literature examining folic acid supplementation in psoriasis patients treated with MTX. After a review of 7 published trials, Al-Dabagh et al (Al-Dabagh, Davis, Kinney, Huang, & Feldman, 2013) reported controversial results. 2 of the 7 trials reported a decreased efficacy of MTX after folic acid supplementation, while 5 of the 7 trials reported the same efficacy with or without supplementation. It's important to highlight that the two studies reporting decreased efficacy, were performed with high-dose MTX (20 mg/week and 5 mg/day). High-dose supplementation may be an aspect that causes a reduced antipsoriatic effect. Even when a few studies suggest the possible loss of efficacy of MTX in psoriasis, due to high-

dose folic acid supplementation, according to the rest of the evidence the authors conclude that, folic acid supplementation should be considered in MTX-treated patients.

In conclusion, the considerations that were taken into account in order to define the administration interval and dose of folate were:

- High doses of folinic acid, more than 5 mg/week, have been associated with a decreased efficacy of MTX as antirheumatic and antipsoriatic drug.
- Coadministration of MTX along with folic or folinic acid have been associated with a decreased efficacy of MTX as antirheumatic drug.
- Folic or folinic acid supplementation between 12 and 48 hours after MTX administration might be preferable, as the available data suggest that dosing at this time frame offers the greatest promise.
- In most of the countries around the world, more than 1000 μg of folic acid contained in a single pharmaceutical dosage form, is considered as a drug product instead of a dietary supplement.

According to the above considerations, the amount per serving of folate in TREXATE Support was established as 1000 μg/day, to be administered 24 hours after the weekly dose of MTX, for 5 days a week. Two examples are shown in figure 9.

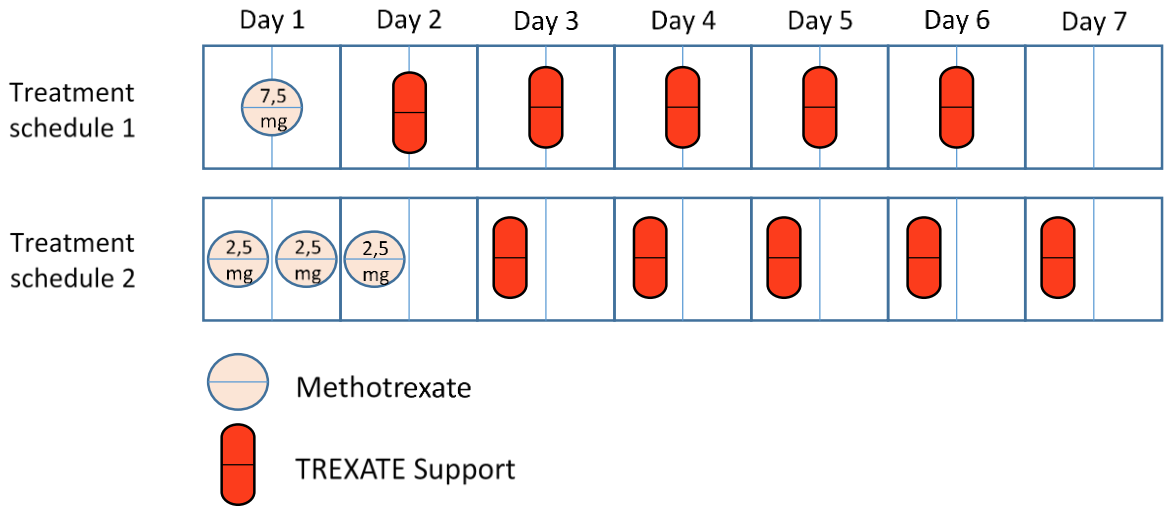

Figure 9. How to use TREXATE Support

7.1.2. Rationale of the selected form of folate (5- methyltetrahydrofolate)

As mentioned in the chapter number 3, folic acid needs to be converted into its active form, THF or reduced form, before exerting its biological action. This conversion is mediated by the enzyme DHFR which is precisely the enzyme that is blocked in patients under treatment with MTX. According to that, in these patients the reaction catalyzed by DHFR is slow and easily reaches saturation, turning folic acid into an inefficient solution for supplementing folate.

In those patients, whose DHFR is inhibited, a reduced form of folate is strongly recommended as the preferred form of supplementation. The available options for reduced forms of folate are folinic acid and 5-methyltetrahydrofolate (5-MTHF).

When a reduced form of folate is used, the intervention of DHFR in order to become the molecule into its active form is no longer required, since the molecule is already presented in its active form, as shown in figure 10.

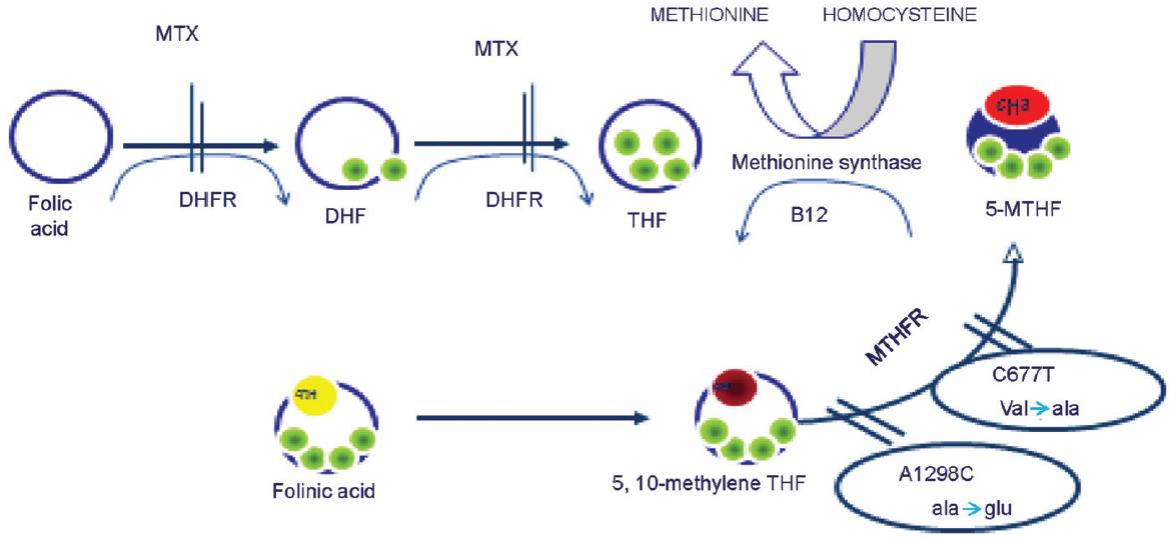

Figure 10. Differences betwen the metabolism of folic acid, folinic acid and 5-MTHF

In several countries around the world, folinic acid is not considered as an approved ingredient for dietary supplements, so the use of a product containing folinic acid would be restricted for those countries. This limitation makes 5-MTHF the best option of folate source in this product.

5-MTHF is a biologically active form of folate and is the most abundant form found in plasma, representing >90% of folate and is the predominant active metabolite of ingested folic acid. Scaglione et al (Scaglione & Panzavolta, 2014) performed a systematic review of the available literature comparing the efficacy and safety of 5-MTHF vs folic acid as a folate source. They conclude that naturally occurring 5-MTHF, as well as being more or at least as effective as folic acid in improving folate status, may present important advantages over synthetic folic acid and, therefore,

supplementation with 5-MTHF may be a valid alternative to folic acid. (Scaglione & Panzavolta, 2014)

Among the advantages of using naturally occurring 5-MTHF instead of synthetic folic acid are:

- It is well absorbed even when gastrointestinal pH is altered and its bioavailability is not affected by metabolic defects,
- reduces the potential for masking haematological symptoms of vitamin B12 deficiency,
- reduces interactions with drugs that inhibit DHFR and overcomes metabolic defects caused by methylenetetrahydrofolate reductase polymorphism, and
- prevents potential negative effects of unconverted folic acid in the peripheral circulation.

Therefore, the use of 5-MTHF instead of folic acid is strongly recommended for external supplementation and food fortification. (Scaglione & Panzavolta, 2014)

Specifically, regarding the use of 5-MTHF in patients taking DHFR inhibitors, Scaglione et al (Scaglione & Panzavolta, 2014) mention: "***There are conditions in which drug treatment causes defects in folate metabolism thus impairing its conversion to the active form. This is the case for treatment with drugs such as MTX, aminopterine, pyrimethamine and trimethoprim which inhibit DHFR. In these conditions, folic acid supplementation is ineffective and folinic acid or 5-MTHF can be a good alternative to folic acid.***"

7.2. Vitamin B12 (as methylcobalamin)

Vitamin B12 serves as an important cofactor in folate metabolism. Vitamin B12 catalyze the conversion of homocysteine into methionine, which is also mediated by one form of THF.

There are at least two clinical trials (Nakagawa, et al., 2006) (Takagi, et al., 2014) supporting the benefit of B12 vitamin, along with folate supplementation, in the reduction of adverse effects associated with the use of pemetrexed.

The amount per serving of 50 µg for vitamin B12 in TREXATE Support was compared to the amounts in the total population of dietary supplements in the US. The comparison was performed by using the Dietary Supplement Label Database of the National Institute of Health, and the findings showed that the content of vitamin B12 in TREXATE Support is above the median. (See figure 11) (NIH Office of Dietary Supplements, 2015)

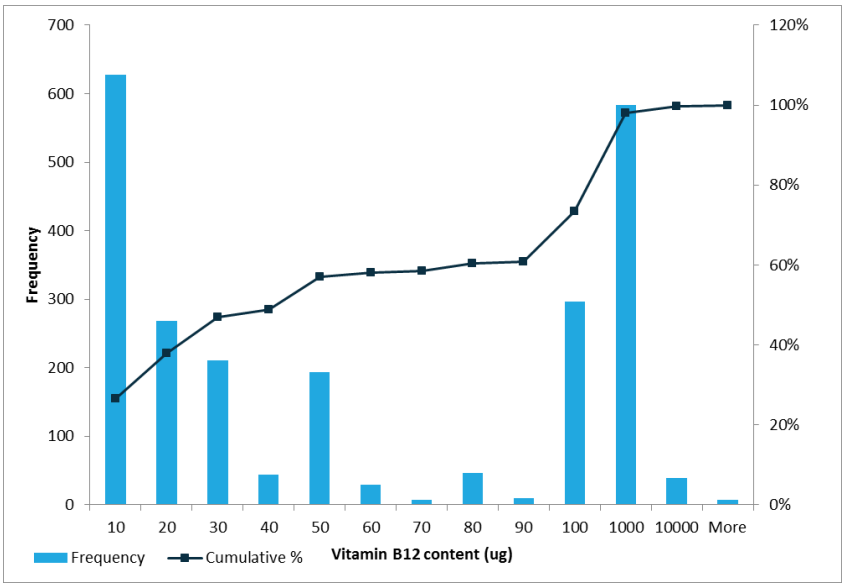

Figure 11. Vitamin B12 content in dietary supplements in the U.S. (unidose dosage forms)

7.3. Vitamin B6 (as pyridoxine hydrochloride):

Vitamin B6 is a cofactor in the metabolism of glycine and serine, which is mediated by a form of THF.

The amount per serving of 10 mg for vitamin B6 in TREXATE Support was compared to the amounts in the total population of dietary supplements in the US. The findings showed that the content of vitamin B6 in TREXATE Support is above the median. (See figure 12) (NIH Office of Dietary Supplements, 2015)

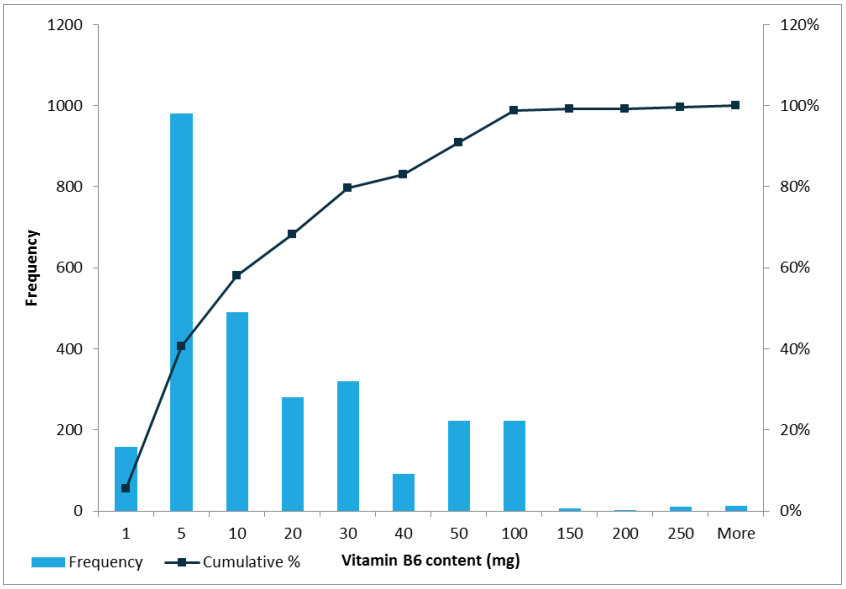

Figure 12. Vitamin B6 content in dietary supplements in the U.S. (unidose dosage forms)

7.4. Vitamin B2 (as riboflavin)

B2 vitamin is another cofactor in folate metabolism.

The amount per serving of 5 mg for vitamin B2 in TREXATE Support was compared to the amounts in the total population of dietary supplements in the US. The findings showed that the content of vitamin B2 in TREXATE Support is above the median. (See figure 13)

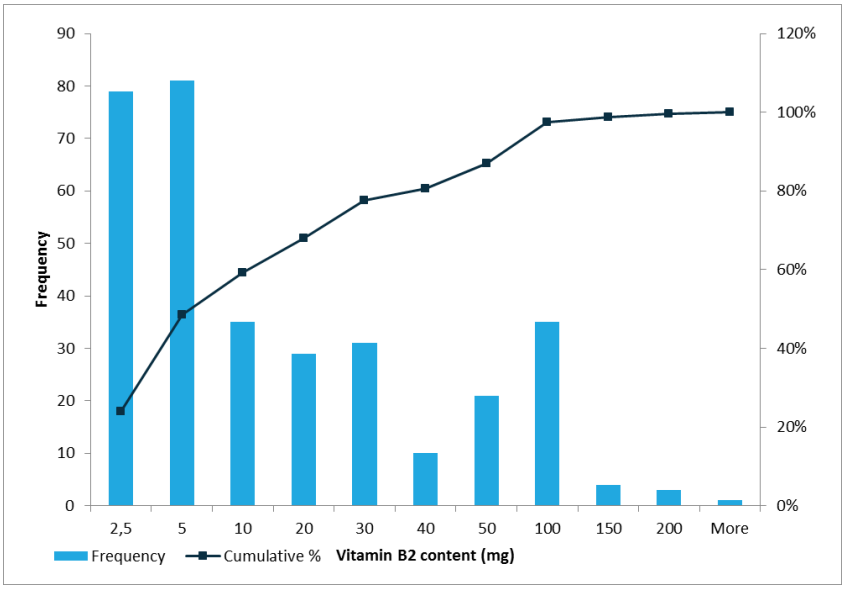

Figure 13. Vitamin B2 content in dietary supplements in the U.S. (unidose dosage forms)

7.5. Milk thistle extract (Silybum marianum) (standardized to 80% silymarin)

Silymarin contributes to reduce the hepatic toxicity of MTX. The hepatic-protective effect of silymarin is widely known in the scientific literature. In addition, there are several pre-clinical trials in which the silymarin has shown to be useful in reducing the hepatic and renal toxicity caused specifically by MTX. (Calegari, et al., 2015) (Ghaffari, Noshad, Ostadi, Ghojazadeh, & Asadi, 2011) (Naik, Chakraborty, Ahmed, Hamza, & Afsal, 2015) (Dabak & Kocaman, 2015)

The amount per serving of 150 mg for milk thistle extract in TREXATE Support was compared to the amounts in the total population of dietary supplements in the US. The findings showed that the content of milk thistle extract in TREXATE Support is above the median. (See figure 14)

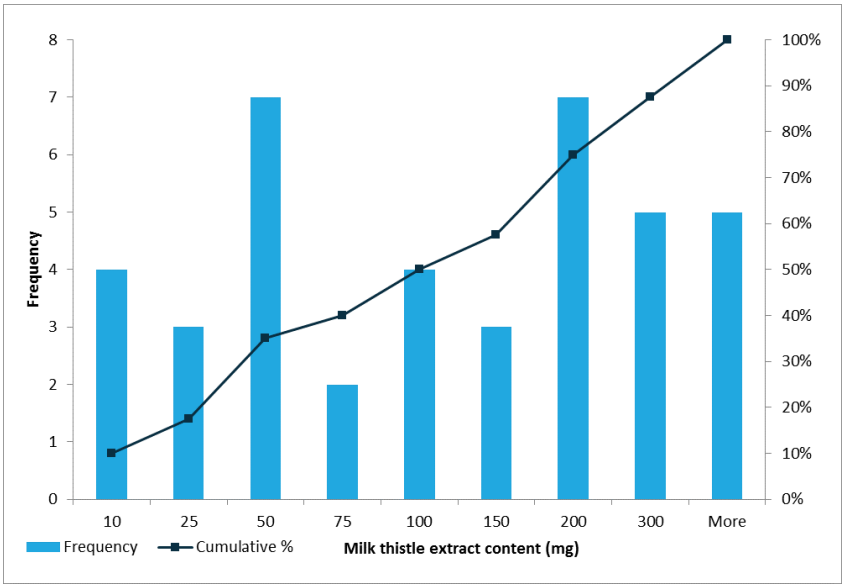

Figure 14. Milk thisthe extract content in dietary supplements in the U.S. (unidose dosage forms)

7.6. L-Methionine

THF is directly involved into the metabolism of methionine, as shown in figure 15. Methionine levels could be affected during a deficiency of folates. (Halsted, et al., 2002)

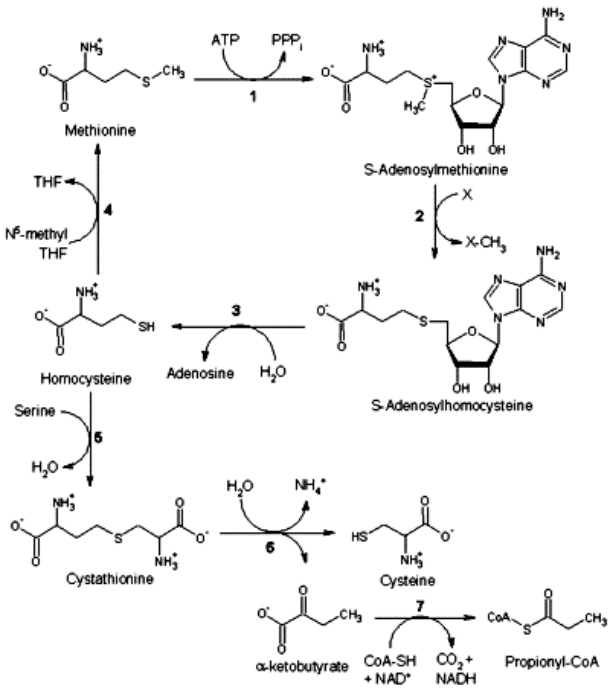

Figure 15. Methionine metabolism

The amount per serving of 50 mg for L-methionine in TREXATE Support was compared to the amounts in the total population of dietary supplements in the US. The findings showed that the content of L-methionine in TREXATE Support is above the median. (See figure 16.)

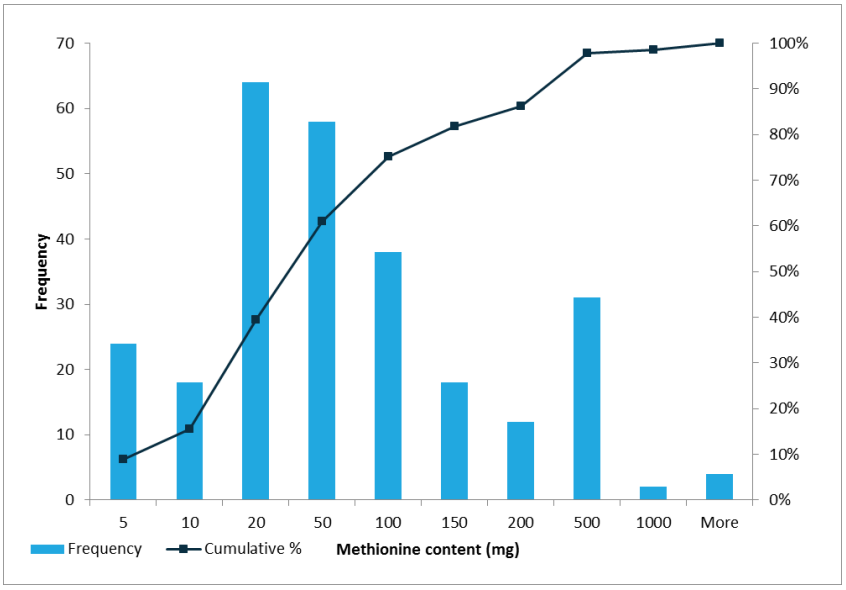

Figure 16. Methionine content in dietary supplements in the U.S. (unidose dosage forms)

7.7. L-Serine and L-Glycine

THF and B6 vitamin are intimately involved in the metabolism of serine and glycine, whose levels could be affected during a deficiency of folates. (See figure 17)

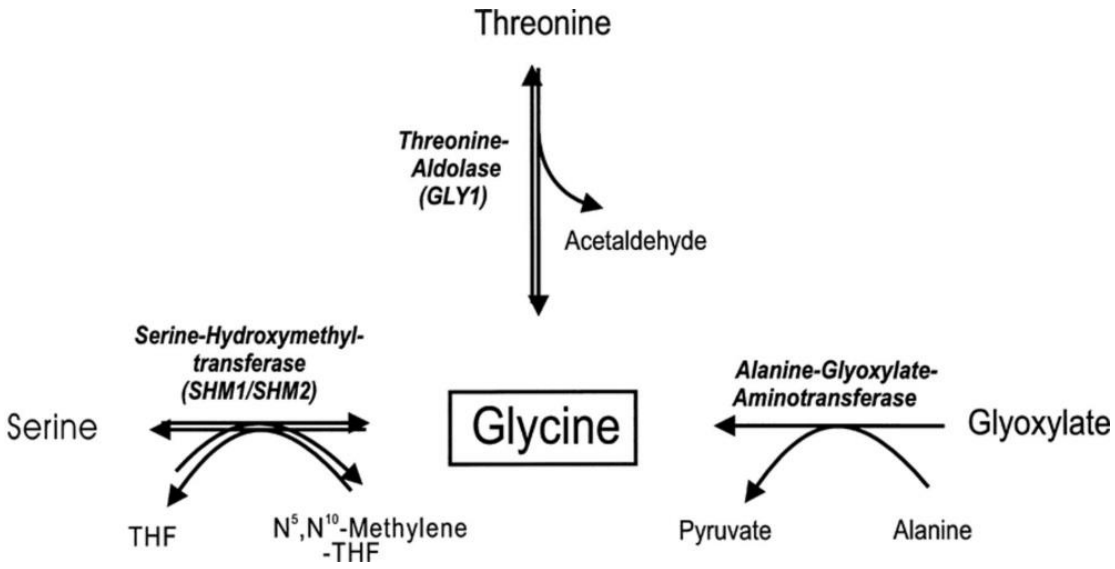

Figure 17. Serine and Glycine metabolism

The amounts per serving of 100 mg for L-serine and L-glycine in TREXATE Support were compared to the amounts in the total population of dietary supplements in the US. The findings showed that the content of L-serine and L-glycine in TREXATE Support are above the median. (See figures 18 and 19)

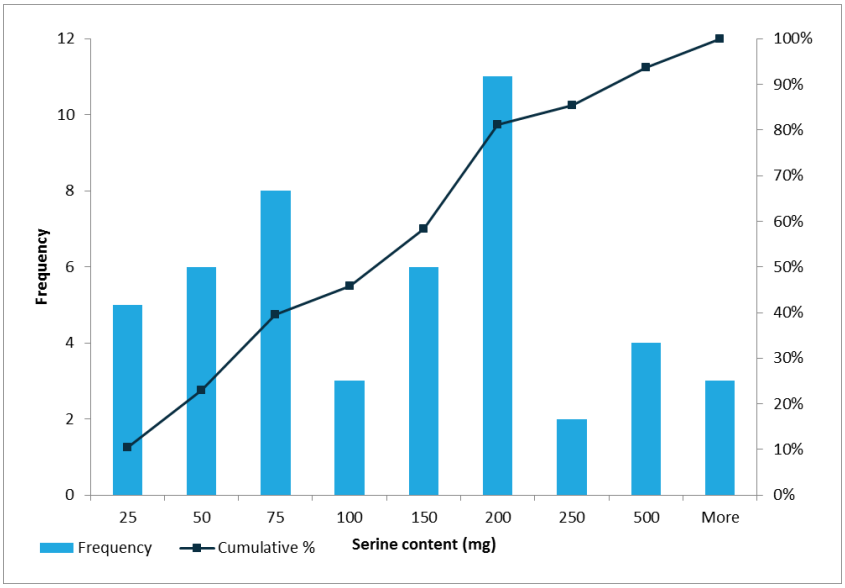

Figure 18. Serine content in dietary supplements in the U.S. (unidose dosage forms)

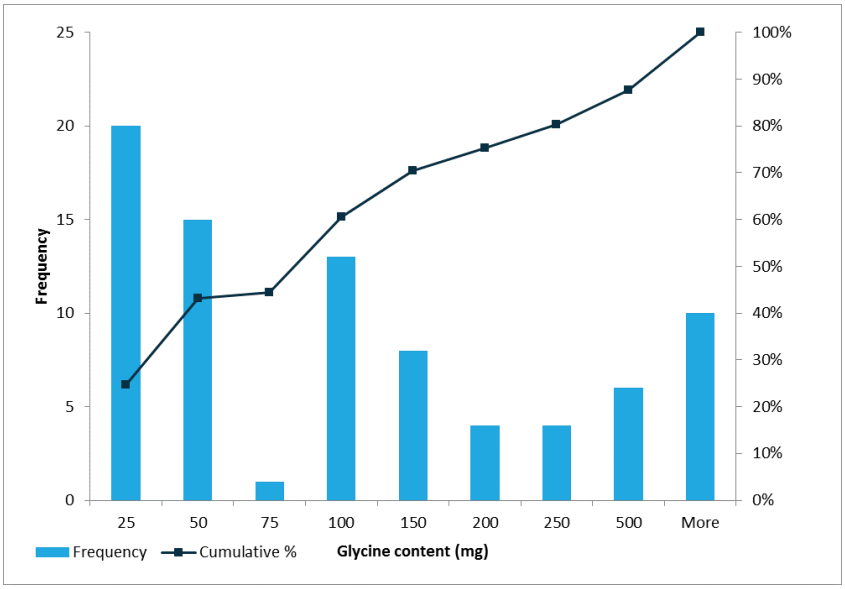

Figure 19. Glycine content in dietary supplements in the U.S. (unidose dosage forms)

8. FACTS AND CLAIMS

8.1. Facts

- Prolonged use of methotrexate may decrease folate stores
- Liver toxicity is a possible side effect caused by the prolonged use of methotrexate

8.2. Claims

- Helps maintain healthy levels of folate
- Promotes healthy liver function

9. PATENT PROTECTION

The patent application was submitted to the USPTO.

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