



## Adverse effect of metformin therapy on serum vitamin B12 and folate: Short-term treatment causes disadvantages?

Lijuan Xu<sup>1</sup>, Zhimin Huang<sup>1</sup>, Xiaoying He, Xuesi Wan, Donghong Fang, Yanbing Li\*

Department of Endocrinology, The First Affiliated Hospital of Sun Yat-sen University, 58th of Zhongshan Er Road, Guangzhou 510080, China

### ARTICLE INFO

#### Article history:

Received 15 October 2012

Accepted 19 May 2013

### ABSTRACT

Diabetes is a global public health challenge that imposes heavy burdens on communities and individuals. Metformin, the first-line medication for diabetes, has the superiority of reducing risk of macrovascular diseases, all-cause mortality and even possibly cancers. Recent observational studies, however, have demonstrated that long-term metformin therapy increases the probability of vitamin B12 and folate deficiency, and might contribute to the progression of diabetic peripheral neuropathy. Despite metformin is widely used and extensively studied, randomized controlled trials performed to explore the effects of metformin on vitamin B12 and folate are limited. Besides, whether short-term treatment causes vitamin deficiency is a pending issue. We postulate that even a few-month treatment with metformin results in the decrease of vitamin B12 and folate. However, supplementation of vitamin B12 rather than the combination of vitamin B12 and folate might be profitable based on the mechanism of metformin on vitamins in patients with type 2 diabetes. This viewpoint differs from those of majority that a combined supplementation of vitamin B12 and folate is inclined to be advised.

© 2013 Elsevier Ltd. All rights reserved.

### Introduction/background

The worldwide prevalence of diabetes has caused a tide of research in the field of diabetic medication and complications. Metformin, an old medication, which is mainly benefiting from the medicine itself and losing body weight it caused, has been widely prescribed to patients with diabetes. Great interest has been roused in recent years for the beneficial effects of metformin on reducing risks of cardiovascular diseases, all-cause mortality and even probably cancers, which is attributed to its roles in regulating AMPK/mTOR pathway [1]. The established advantages and safety of metformin have made it the first-line treatment in patients with type 2 diabetes.

However, some studies have demonstrated that long-term use of metformin combined with other antidiabetic agent increased the risk of vitamin B12 and folate deficiency, and thus influenced the homocysteine metabolism and contributed to the progression of diabetic peripheral neuropathy [2]. But controversies remain regarding the influence of metformin on folate. One clinical trial from Netherland reported that metformin combined with insulin lowered both folate and vitamin B12 levels, and simultaneously increased homocysteine concentration compared with insulin therapy alone [3]. But another cross-sectional study demonstrated that patients treated with metformin had no significant depletion

of serum folate [4]. And still another trial showed that metformin decreased folate levels, but after adjustment for body mass index and smoking, the decrement turned out insignificant [5].

The proposed mechanism of metformin-related vitamin B12 deficiency might be its competitive inhibiting effect on vitamin B12 absorption, altering enterokinesia, ileal morphological structure, bacterial flora, the levels of intrinsic factor and the vitamin B12-intrinsic factor complex [6]. Since both vitamin B12 and folate participate in the metabolism of homocysteine through methylation pathway, we speculate that the decrease of folate levels might be an indirect outcome of metformin acting on vitamin B12, which seems to explain the inconsistent effects of metformin treatment on folate in the clinical trials we mentioned before.

Homocysteine, an intermediate product of methionine converting to cysteine, is metabolized via the following pathways: (1) remethylation, in which homocysteine obtains a methyl group from 5-methyltetrahydrofolate and turns back into methionine with the help of coenzyme vitamin B12. (2) Transulphuration, in which homocysteine forms cystathionine after serine molecule attachment with the aid of vitamin B6. (3) Reconversion, in this process a part of homocysteine reconverts into S-adenosylhomocysteine (SAH) [7,8]. In the first two pathways, folate, vitamin B12 and vitamin B6 are indispensable for homocysteine metabolism, and supplementation of those vitamins can reduce the levels of homocysteine.

Since 1969, McCully found homocysteine might have close association with atherosclerosis [9]. After that, an increasing number of studies have explored the relations between homocysteine

\* Corresponding author. Tel./fax: +86 20 28823390.

E-mail address: [easd04lyb@126.com](mailto:easd04lyb@126.com) (Y. Li).

<sup>1</sup> These authors contributed equally to this paper.

and cardiovascular disease, cerebrovascular disease and diabetes. Quantitative studies showed that hyperhomocysteinemia was an independent risk factor for arteriosclerotic vascular diseases. The odds ratio (OR) for coronary artery disease of a 5  $\mu\text{mol/L}$  homocysteine increment is 1.6 (95% confidence interval [CI], 1.4–1.7) for men and 1.8 (95% CI, 1.3–1.9) for women [10]. In patients with established coronary heart diseases, a small gradient in the extent of atherosclerosis was present with increasing of homocysteine ( $r = 0.25$ ,  $P < 0.05$ ) [11]. Some other observational studies demonstrated that homocysteine was closely associated with the risk of developing stroke, peripheral vascular disease, diabetes and its complications. Minor to moderate hyperhomocysteine already played a role in these chronic diseases and its level predicted mortality independent of age, gender and blood pressure [12,13].

Surprisingly, several large trials, such as the Western Norway B-vitamin intervention trial (WENBIT), the Norwegian vitamin (NOR-VIT) trial, the vitamin intervention for stroke prevention (VISP) trial, the study of the effectiveness of additional reductions in cholesterol and homocysteine (SEARCH) and the women's antioxidant and folic acid cardiovascular study (WAFACS) suggested that vitamin B6 and/or combination of vitamin B12 and folate supplementation were invalid or even unfavorable for the prognosis of cardiovascular and cerebrovascular diseases [14–18]. One potential explanation might be that the implementation of fortification of cereals with folate in some countries to reduce neonatal neural tube defects has covered up the effect of B-vitamins supplementation. Another possible reason was that although homocysteine was found related to cardiovascular and cerebrovascular diseases, it might act merely as an accompaniment rather than a risk factor. It has been suggested that SAH, the precursor of homocystiene, might be a more sensitive indicator of cardiovascular disease. Vitamin supplement that reduces homocysteine concentration may have no effect on SAH level. Thus, Vitamin B supplement therapy aiming to improve homocysteine level is unlikely to be eligible in preventing cardiovascular events.

Different from the above large clinical trials, Mashavi et al. found that supplementation of vitamin B12, folate and vitamin B6 improved small arterial elasticity in metformin treated diabetic patients [19]. We speculate that the difference between these studies derives from the probability that metformin therapy might lower vitamin B12 and the subsequent folate levels, so supplementation in that study with vitamin B12, folate and vitamin B6 resulted in positive effects. In contrast, patients enrolled in those large clinical trials who were not treated with metformin did not generally suffer from B-vitamins deficiency, therefore exhibited no further benefits.

### The hypothesis

We noticed that limited studies conducted so far were mostly concerning long-term treatment with metformin combined with other antidiabetic therapies on B-vitamins. One clinical trial explored the adverse effect of six-week metformin therapy on serum concentrations of vitamin B12, folate and homocysteine [20]. But in this trial metformin was compared with rosiglitazone rather than placebo or blank control, which made the conclusion that short-term metformin decreased vitamin B12 levels assailable. Whether short-term administration of metformin reduces vitamin B12 as well as folate levels is yet uncertain. On this purpose, we performed a pilot study exploring the effect of short-term metformin on serum vitamin B12 and folate concentrations. A total of 40 newly diagnosed type 2 diabetic patients were recruited and randomized into two groups. To minimize the interference of other hypoglycemic medications on vitamin B12 and folate, two-week continuous subcutaneous insulin infusion (CSII) was provided. Patients in Group One received treatment of metformin 0.5 g, three

times a day plus CSII, while the other group received CSII treatment alone. Short-term intensive insulin regimen was similar in the two groups that maintained for 2 weeks and then suspended. Patients in Group One continued with metformin for a total of 3 months, while those in Group Two maintained glycemic control with diet and exercise alone after CSII suspension. As a result, we found that three-month therapy of metformin had a trend of reducing vitamin B12 and folate concentrations. But due to the small sample size of patients enrolled, the changes were not statistically significant.

Although some studies have showed that supplementation of vitamin B12, folate and vitamin B6 were beneficial in patients treated with metformin, the potential worry still exists on the basis of series of large-scale studies we mentioned before that combination treatment with vitamin B12, folate and/or vitamin B6 brought no further profits. In the light of metformin pharmacological mechanism, we deduce that folate reduction might be the indirect outcomes of vitamin B12 deficiency. Under this circumstances, we infer that supplementation of vitamin B12 alone is a better choice than the combination therapy of vitamin B12 and folate.

In this context, we hypothesize that three-month treatment with metformin reduces vitamin B12 and folate concentrations in patients with type 2 diabetes. Supplementation of vitamin B12 alone rather than combination of vitamin B12 and folate might be sufficient to improve the disorders. Long-term treatment with metformin probably contributes to the progression of diabetic peripheral neuropathy, so early supplementation with vitamin B12 is likely to be advantageous.

### Discussion

This hypothesis provides the basis to detect the adverse effect of metformin on vitamin B12 and folate levels. We find that even short-term treatment tends to reduce vitamin B12 and folate concentrations in patients with type 2 diabetes. For the best we know, our pilot study firstly used 2-week CSII to well control glucose and then suspended, which avoided the influence of other medication to a great extent and made our postulation that 3-month metformin therapy could cause disadvantages more dependable. Following this, early supplementation of vitamin B12 and folate might be appropriate. However, metformin directly influences the ingestion and absorption of vitamin B12 and might indirectly act on folate metabolism. Besides, folate is proven effective only in the prevention of neonatal neural tube defects and in the treatment of certain diseases such as megaloblastic anemia. No further convinced advantageous evidences were found for its supplementation. Thus in our hypothesis, vitamin B12 monotherapy other than B-vitamins combined therapy is thought to be sufficient for those who are on metformin treatment. This viewpoint differs from those of majority that a combined supplementation of vitamin B12 and folate is inclined to be advised. Our postulation and the upcoming clinical trial will contribute to the clinical treatment as well as the research and development of compound medicines.

### Conflict of interest

None declared.

### Support

This work was supported by Guangdong Pharmaceutical Association Research Fund (no. 2012C09).

### References

- [1] Rocha GuilhermeZ, Dias MaríliaM, Ropelle EduardoR, et al. Metformin Amplifies Chemotherapy-Induced AMPK Activation and Antitumoral Growth. *Clin Cancer Res* 2011;17(12):3993–4005.

- [2] de Jager Jolien, Kooy Adriaan, Lehert Philippe, et al. Long term treatment with metformin in patients with type 2 diabetes and risk of vitamin B-12 deficiency: randomized placebo controlled trial. *BMJ* 2010;340:c2181.
- [3] Wulffélé MG, Kooy A, Lehert P, et al. Effects of short-term treatment with metformin on serum concentrations of homocysteine, folate and vitamin B12 in type 2 diabetes mellitus: a randomized, placebo-controlled trial. *J Intern Med* 2003;254(5):455–63.
- [4] Pongchaidecha M, Srikusalanukul V, Chattananon A, Tanjariyaporn S. Effect of metformin on plasma homocysteine, vitamin B12 and folic acid: a cross-sectional study in patients with type 2 diabetes mellitus. *J Med Assoc Thai* 2004;87(7):780–7.
- [5] de Jager J, Kooy A, Lehert P, et al. Long term treatment with metformin in patients with type 2 diabetes and risk of vitamin B-12 deficiency: randomised placebo controlled trial. *BMJ* 2010;340:c2181.
- [6] Adams JF, Clark JS, Ireland JT, Kesson CM, Watson WS. Malabsorption of vitamin B12 and intrinsic factor secretion during biguanide therapy. *Diabetologia* 1983;24:16–8.
- [7] Finkelstein JD, Martin JJ. Methionine metabolism in mammals: adaptation to methionine excess. *J Biol Chem* 1986;261:1582–7.
- [8] Luo X, Xiao Y, Song F, Yang Y, Xia M, Ling W. Increased plasma S-adenosyl-homocysteine levels induce the proliferation and migration of VSMCs through an oxidative stress-ERK1/2 pathway in apoE(-/-) mice. *Cardiovasc Res* 2012;95(2):241–50.
- [9] McCully KS. Vascular pathology of homocysteinemia: implications for the pathogenesis of arteriosclerosis. *Am J Pathol* 1969;56(1):111–28.
- [10] Boushey CJ, Beresford SA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. *JAMA* 1995;274(13):1049–57.
- [11] Montalescot G, Ankri A, Chadeaux-Vekemans B, et al. Plasma homocysteine and the extent of atherosclerosis in patients with coronary artery disease. *Int J Cardiol* 1997;60(3):295–300.
- [12] Stehouwer CD, Gall MA, Hougaard P, Jakobs C, Parving HH. Plasma homocysteine concentration predicts mortality in non-insulin-dependent diabetic patients with and without albuminuria. *Kidney Int* 1999;55(1):308–14.
- [13] Rasouli ML, Nasir K, Blumenthal RS, Park R, Aziz DC. Plasma homocysteine predicts progression of atherosclerosis. *Atherosclerosis* 2005;181(1):159–65.
- [14] Øyvind Bleie, Helga Refsum, et al. Changes in basal and postmethionine load concentrations of total homocysteine and cystathionine after B vitamin intervention. *Am J Clin Nutr* 2004;80:641–8.
- [15] Kaare Harald Bønaa, Inger Njølstad, Per Magne Ueland, et al. Homocysteine Lowering and Cardiovascular Events after Acute Myocardial Infarction. *N Engl J Med* 2006;354:1578–88.
- [16] Viswanathan A, Raj S, Greenberg SM, et al. Plasma Aβ, homocysteine, and cognition: The Vitamin Intervention for Stroke Prevention (VISP) trial. *Neurology* 2009;72:268–72.
- [17] Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine (SEARCH) Collaborative Group. Effects of Homocysteine-Lowering With Folic Acid Plus Vitamin B12 vs Placebo on Mortality and Major Morbidity in Myocardial Infarction Survivors. *JAMA* 2010;303(24):2486–94.
- [18] Christine M, Albert Nancy R, et al. Effect of folic acid and B-vitamins on risk of cardiovascular events and total mortality among women at high risk for cardiovascular disease: a randomized trial. *JAMA* 2008;299(17):2027–36.
- [19] Mashavi M, Hanaha R, Boazc M, et al. Effect of homocysteine-lowering therapy on arterial elasticity and metabolic parameters in metformin-treated diabetic patients. *Atherosclerosis* 2008;199:362–7.
- [20] Sahin Mustafa, Tutuncu Neslihan B, et al. Effects of metformin or rosiglitazone on serum concentrations of homocysteine, folate, and vitamin B12 in patients with type 2 diabetes mellitus. *J Diabetes Complicat* 2007;21:118–23.



本文献由“学霸图书馆-文献云下载”收集自网络，仅供学习交流使用。

学霸图书馆（www.xuebalib.com）是一个“整合众多图书馆数据库资源，提供一站式文献检索和下载服务”的24小时在线不限IP图书馆。

图书馆致力于便利、促进学习与科研，提供最强文献下载服务。

#### 图书馆导航：

[图书馆首页](#)   [文献云下载](#)   [图书馆入口](#)   [外文数据库大全](#)   [疑难文献辅助工具](#)