Author's response to reviews

Title: Metformin use in patients with type 2 diabetes mellitus is associated with reduced risk of deep vein thrombosis: A Non-randomized, Pair-matched Cohort Study

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Author's response to reviews: see over
Dear Editors:
Thank you for your letter on September 22, 2014 containing the reviewers’ comments on our manuscript (MS: 2014867863134212) entitled "Metformin use in patients with type 2 diabetes mellitus is associated with reduced risk of deep vein thrombosis: A Non-randomized, Pair-matched Cohort Study" by Dai-yin Lu, et al. The comments of the reviewers are appreciated and have been helpful for us during the revision of our manuscript. We have also attempted to answer each of the questions raised and offer point-by-point responses to the comments of the reviewers. All corrections were presented with underline. Thank you for your consideration of our article.

Sincerely yours,

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Reply to Reviewers’ Comments

The authors wish to thank the reviewers’ very constructive comments! We have addressed every point the reviewers raised by updating new concepts (focusing on risk factors venous thromboembolism) and discussed these important issues in the revised manuscript.

Reviewer #1

Reviewer’s report:

Minor essential revisions:

1. In the Introduction, please explain the rationale of the study more clearly.

Responses:

Thank you for this important comment. We have revised our manuscript to describe the study purpose more clearly. [Page 6, Paragraph 1, Line 16]

2. In the Conclusions, please state the effect of metformin in a less positive way, since this is the first evidence only.

Responses:

Thank you for the comment. We have revised our description accordingly. [Page 21, Paragraph 2, Line 2]
Reviewer #2

Reviewer’s report:

1. In the abstract section, the results (page 4, line 17) describe that “…44(0.6%) from the cohort with metformin and 16(0.21%) from the control group…”. The prevalence of DVT is higher in the metformin group than that in the non-metformin control group, how can the subjects with metformin use would experience a risk reduction in the development of DVT? Furthermore the prevalence rate of DVT in the context of “Results” (page 12, line 13 and 14) is a little bit different from those described in the abstract.

Responses:

Our analysis showed that of the 14945 patients (7167 patients with metformin vs. 7778 control), 60 (0.40%) patients developed DVT during a mean follow-up period of 3.74 years, including 16 (0.21%) from the cohort with metformin and 44 (0.56%) from the control group. We are sorry for the misleading error in typewriting and have corrected the descriptions. (Page 4, Paragraph 3, Line 15)

2. In the context of discussion (page 18, line 8-9) it is described that “…metformin may provide endothelial protection to reduce thromboembolic event…” The endothelial protection is more likely to reduce the arterial thrombosis event, e.g. CAD, stroke and PAOD, rather than to reduce the DVT event which is more blood flow and coagulation-related. The author spent too much time to discuss the mechanism to reduce the cardiovascular event not the DVT event.

Responses:

Thank you for this important comment. Hyper-coagulation is indeed an essential part in development of venous thrombosis. Previous studies have demonstrated patients with insulin resistance had impaired coagulation. The beneficial effect of metformin on coagulation factors have been identified previously. In patients with metformin therapy, factor XIII antigen and activity levels in vivo were reduced over a 12-week period.(1) Peter et al indicated that metformin therapy in subjects with type 2 diabetes mellitus causes a fall in factor VII antigen concentrations that is independent of the dosage employed and that persists for at least 6 months.(2) A study of 2368 patients also demonstrated that treatment with insulin-sensitizing treatment strategy, primarily metformin in type 2 diabetes mellitus and documented coronary artery
disease led to lower plasminogen activator inhibitor type 1 antigen, tissue plasminogen activator antigen, and lower fibrinogen at all intervals after baseline,(1-3), suggesting the benefit effect of metformin in reducing the hyper-coagulation in diabetic patients. We have made more discussion about the effects on coagulation function of metformin according to reviewer’s suggestion (Page 16, Paragraph 3, Line 16)

In addition to the benefits of endothelial function, metformin also increases carotid systolic blood flow,(4) peripheral blood flow and glucose uptake.(5)

Besides hyper-coagulation, endothelial dysfunction played an important role in the formation of venous thrombosis. Vascular endothelial cell surface is the major site of control of these coagulant and anticoagulant interactions. Mazzoccoli et al revealed patients with DVT showed a significant decrease of flow-mediated dilation, a marker of endothelial dysfunction, compared with patients without DVT, suggesting the importance of endothelial dysfunction in the pathogenesis of DVT.(6) On the other hand, metformin therapy in patients with type 2 diabetes mellitus improved endothelial function: metformin is associated with improvement in some markers of endothelial function.(7) Subjects who received metformin had significant improvement in acetylcholine-stimulated flows compared with those treated with placebo.(8)

3. In the study of co-morbidities, the author included mostly the cardiovascular risk factors, e.g. hypertension, coronary artery disease, hyperlipidemia, etc. The other important venous thromboembolic risk factors, e.g. cancer, surgery, immobilization, pregnancy, presence of antiphospholipid antibodies, myeloproliferative neoplasm or primary thrombophilia were unfortunately not included. These defects may cause serious bias.

Responses:
Thank you for your comments and we have already added information including underlying malignancy, fracture, and major operation. Patients with DVT had more co-morbidities of cancer, including solid tumors and hematologic malignancies, and fractures. They also underwent more major operations, defined as cardiothoracic, abdominal, pelvic surgery and orthopedic surgeries of lower limbs. Furthermore, the information of hormone replacement was also included. After these predisposing risk factors are accounted for, metformin still has independent predicting power toward lower risk of DVT. We had added these parameters in the tables and revised the discussions in our manuscript. (Page 13, Paragraph 2, Line 4; and Page 31, Table 3,
4. Hyperhomocysteinemia is known to be associated with an increased risk of arterial and venous thrombosis. Hyperhomocysteinemia usually occurs when there are a MTHFR enzyme C677T homozygous mutation, which is found to present in about 7-10% of Taiwanese population, and low plasma level of folate, vitamin B6 or vitamin B12 coexist. Long term use of metformin has been shown to be able to induce vitamin B12 deficiency in 10 to 30% patients. Therefore metformin use in patients with type 2 DM may cause B12 deficiency and homocysteinemia may occur when there is a MTHFR C677T homozygous mutation. Therefore metformin long-term use in type 2 DM may be associated with increased, not reduced risk of DVT.

Responses:
Thank you for your comments and we completely agree with your concern about the issue of vitamin B12 and metformin. Previous studies reported metformin treatment was associated with a decrease in vitamin B12 concentration,(9) which was present in 5.8% of the population (10) and vitamin B12 deficiency may be associated with hyperhomocysteinemia. However the association between vit B12 deficiency and deep vein thrombosis remained undetermined. Previous data have suggested that there is no adequate evidence concerning the role of metformin therapy and hyperhomocysteinemia. A previous study from Thailand demonstrated that although metformin may have caused low vitamin B12 levels, there were no significant changes to homocysteine levels.(11) Hoogeveen et al further found that metformin-exposed patients had only slightly higher serum total homocysteine levels then control group.(12, 13) Furthermore, the dose-response relationship between cumulative exposure to metformin and total homocysteine level was not identified.(13) Besides medications, other dietary factors such as fruit and vegetable consumption in diabetic patients could also be strong independent determinants of homocysteine levels.(14) Recent studies show that lowering homocysteine levels does not decrease the risk for atherosclerosis or thrombosis.(15, 16) This supports the theory that homocysteine may just be an "innocent bystander" and not the cause of these conditions. Therefore, large-scale prevention studies identifying high-risk patients through genetic tests, like C677T homozygous mutation targeting on populations with
low folate intake have not been performed. (17)

In addition, it has been reported that serum vitamin B12 levels do not adequately assess tissue vitamin B12 stores. (18) Patients with type 2 diabetes may show normal extracellular vitamin B12, but disturbed intracellular B12-dependent biochemical reactions. Metformin treatment was associated with low serum vitamin B12 level, while improved intracellular vitamin B12 metabolism despite low serum vitamin B12. (19)

Furthermore, as we mentioned above, metformin may provide additional benefit in reduction of hyper-coagulation and endothelial function improvement. (1-3, 7) It can also increase the blood flow. (4, 5, 20) Because of so many benefits, current American Diabetes Association (ADA) guideline suggested metformin should be used a first choice for type 2 diabetic patients. (21) We also added the discussion about the association between metformin, vitamin B12 deficiency and hyperhomocysteinemia into our manuscript. (Page 18, Paragraph 2, Line 13)
References


