PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Meta-analysis: The effects of smoking on the disposition of two commonly used antipsychotics, olanzapine and clozapine
AUTHORS	Saruwatari, Junji; Tsuda, Yoshiyuki; Yasui-Furukori, Norio

VERSION 1 - REVIEW

REVIEWER	Massimo Carlo Mauri Clinical Psychiatry Clinical Neuropsychopharmacology Unit IRCCS Ospedale Maggiore Policlinico Clinical Neuropsychopharmacology
REVIEW RETURNED	22-Oct-2013

GENERAL COMMENTS	It is a well assessed meta-analysis on the effects of smoking on the disposition of two antipsychotics, olanzapine and clozapine although it is a well known factor influencing drug plasma levels. In my opinion there are no technical errors or inaccuracies and no important informations have been omitted. Some comments: Table 1 and 2: can you indicate the applied diagnostic criteria, if available (i.e. ICD-9, DSM IV). About clozapine it is not clear if the Authors consider the sum of plasma levels of mother drug and its metabolite N-clozapine or only clozapine levels. It should be interesting to know the ratio CLZ/N-CLZ levels as an index of metabolic activity of patients. Authors should conclude and suggest more clearly what is the indicative oral dose adjustement of clozapine and olanzapine in smoking patients on the basis of collected data.

REVIEWER	Cand.pharm. PhD Tore Haslemo Center for Psychopharmacology Diakonhjemmet Hospital Oslo, Norway
REVIEW RETURNED	04-Nov-2013

GENERAL COMMENTS	There is clearly an interest in this field, and a meta-analysis like this is welcome.

	I have myself submitted data from two different papers to this study, and therefore has some knowledge on how the authors have collected data. One of my previous studies had relevant data on both olanzapine and clozapine, and I submitted data for both of these (clozapine n=33, olanzapine n=40). In the meta analysis the authors only included the clozapine-data. I can not find any information or criteria in the manuscript of this practice, but it might be that there were already "enough" data on olanzapine, and thereby only including clozapine-data. Recent large TDM-studies are not mentioned in the manuscript. These are of interest since they allow for correction of several factors by using different statistical approach through multiple regression/linear mixed model, These studies could not be included in the meta-analysis, but could have discussed to relate the effect of smoking to other factors.. **General comments:** The authors have conducted a meta-analysis to estimate the effects of smoking on dose-adjusted serum concentrations of clozapine and olanzapine. The aim was to clarify these effects, and create standards for adjusting dose in clinical practice. **Assessing current knowledge on this field to create to practical dosing guidelines is clearly needed. The study problem is relevant, the methods are adequate, and the manuscript is clearly written.** However the manuscript is lacking some information about the studies that could not be included. The authors state that 42 studies included data on serum concentrations and smoking habits, but 11 olanzapine studies and 18 clozapine studies could not be included. Please state the reason for this, and update Figure 1 with more details on number of studies. Were data from all included studies confirmed by the original authors? Did they not respond? The authors might comment on the size of this meta-analysis, seen in light of the quality and limited number of included studies (4 and 7 for clozapine and olanzapine respectively). The data material for this meta-analysis is comparable to some of the largest TDM-studies published on this field. Do the authors find it sufficient to search Medline, and not Embase, for the selection of studies for inclusion in this meta-analysis? Cigarette smoking has such an important effect on the

	pharmacokinetics of these two drugs, that conducting these studies without correcting for other variables might be defended. But in recent years there have been other studies that included several other factors of importance, like co-medication, patient age, gender, ethnicity and genotype (CYP1A2 and UGT1A4). Although the manuscript now clearly state that this is a study of the effect of smoking only, some of these factors could be discussed more in detail or at least be treated more equally. This manuscript might therefore benefit from removing the additional meta-analysis of factors race and sex, and more clearly sticking to the aim of making practical clinical standards for dose adjustments of clozapine and olanzapine. These findings should be communicated more clearly, also in the abstract, with practical dosing advice rather than solely the observed mean differences. The findings seem valid, but the authors should stick to the primary objective of the study. **Specific comments:** **Abstract** Abstract should include alternative explanations of the observed results, rather than only mean differences ((ng/ml)/ml/day). Key message: The authors state that 58-88% of patients are smokers, this implies that the vast majority of patients are smokers. Dose correction for smoking might therefore be performed through lowering doses in non-smokers rather than increasing doses in cigarette smokers. Dose reduction (per cent) in non-smokers might be a more suitable practical guideline? **Introduction** Page 8 line 24. *"Previous clinical studies reported that smokers had an approximately five-fold lower dose corrected steady-state plasma*

	olanzapine concentration." Does this represent the current view of the effect of cigarette smoking on olanzapine? This reference (reference 8 Carrillo et al.) is based on a very small study (n=17). **Results:** Page 13 line 46: *Since we could not obtain the data regarding the olanzapine disposition during the condition of smoking cessation….* Was it considered part of the aim of this study to determine the effect of smoking cessation on C/D ratios of olanzapine? This kind of data might be available from a few published case reports, but is probably not available in a large scale. Page 14, line 50: *Four studies regarding the clozapine disposition met our criteria,* all of these were retrospective studies. Page 16, line 12-24 could be removed after stating that all clozapine studies were retrospective (page 14, line 50). **Discussion** Page 19, line 56: *Two and four studies are included for additional meta-analysis of sex and race, and only for olanzapine.* Bigos et al. and Citrome et al. might cover and discuss these topics better in their respective papers. Covering these topics are considered outside the scope of this paper, at least when omitting several other important factors. Page 19 line 35: Other factors affecting the disposition of clozapine and olanzapine. Co-medication should also be discussed here. Page 20 line 35: Limitations of the study: The inclusion process should be covered more in detail in this manuscript, both in Figure 1 and in the text. Other patient factors might be covered better: Studies performed in specific patient populations (elderly, women, different diagnostic groups) might lead to increased or reduced impact of smoking. Several large studies in the recent years covered

	relevant factors like drug interactions, sex, age, race and genetics. . Page 22, line 15: The conclusion of the study does not reflect the aim: "*create standards to adjust doses based on smoking status*". The conclusion of the study should not request additional research, but rather give practical advice based on the included studies. **Figures** Figure 1 Flow chart (more details regarding the process of inclusion/exclusion are needed) Figure 2 Forest plot OLA (n=7) Try to include information from figure 3 and 4 in figure 2. Figure 3 Forest plot prospective OLA (incorporate information about study type in Figure 2) Figure 4 Forest plot retrospective OLA (incorporate information about study type in Figure 2) Figure 5 Forest plot clozapine Supplementary figure 1: Funnel plot olanzapine (not stated that Citrome et al. are represented with 3 data points in this figure) Supplementary figure 2: Funnel plot clozapine (n=4) Supplementary figure 3: Race (not sex as stated in figure legends) Supplementary figure 4: Sex (not race as stated in figure legends)

REVIEWER	Adrian Barnett Queensland University of Technology, Australia
REVIEW RETURNED	20-Nov-2013

GENERAL COMMENTS	The three results from Citrome are treated as independent in the meta-analysis, but this is unlikely to be true. It would be better to include a random intercept in the mixed effect meta-analysis to acknowledge the correlation in results from the same study. This may widen the overall confidence interval. Are there any caveats about extending these doses from a meta-analysis to the patient setting? Confounding was not really addressed. Other associations with age and sex are mentioned, but are these confounders of cigarette smoking? Cigarette smoking is often associated with a number of other risky behaviours such as alcohol consumption. Would alcohol consumption effect these drugs?

	Minor comments - Article focus "based on smoking status" no "the" - Page 7 Line 38 - "shows a" missing a space - Page 9 line 41, the year 1946 seems very early, when were these drugs first available? I note the earliest publication date of included studies is 2008. - Page 10 line 18, did all subjects need to take the drug for a week? What if only 90% complied with taking the drug for a week or more? - Page 10 line 44, did any authors not respond to the change of unit request? I see that this information appears later on page 20, but it would be worth flagging here that not all authors responded. - Table 1, is the age for the Citrome et al study the range? (same comment for Weide study in Table 2) - Page 13 line 41, 'No significant publication bias' - The planned subgroup analysis should be mentioned in the methods. - Page 17, spell-out BPRS (and page 18) - Page 17 line 35, 'association' not 'correlation' - Page 20, the simple definition of smokers should be mentioned in the methods section and/or introduction

VERSION 1 – AUTHOR RESPONSE

Authors' responses (Re) to the comments (C) from REVIEWER #1 (Dr. Massimo Carlo Mauri)

C1. Table 1 and 2: can you indicate the applied diagnostic criteria, if available (i.e. ICD-9, DSM IV).

Re: As suggested by the reviewer, we added the applied diagnostic criteria to Tables 1 and 2.

C2. About clozapine it is not clear if the Authors consider the sum of plasma levels of mother drug and its metabolite N-clozapine or only clozapine levels. It should be interesting to know the ratio CLZ/N-CLZ levels as an index of metabolic activity of patients.

Re: As pointed out by the reviewer, we could not consider the sum of the plasma levels of its metabolite, norclozapine, nor the ratio of norclozapine/clozapine. This meta-analysis used only the clozapine levels for two reasons: (1) to allow us to include as many studies as possible and (2) to develop simple standards that could be used in clinical practice. In order to address the reviewer's comments, we added the sentences as limitations in the Discussion section in the revised manuscript: "In previous studies, the sum concentrations of clozapine and its metabolite, norclozapine, and the norclozapine to clozapine ratio, were also used as a clinical outcome and an index of metabolic activity, respectively. However, we could not use these parameters for the present meta-analysis, because we used only the clozapine concentration to dose ratio in order to be able to include as many studies as possible and to develop simple standards that can be used in clinical practice."

C3. Authors should conclude and suggest more clearly what is the indicative oral dose adjustment of clozapine and olanzapine in smoking patients on the basis of collected data.

Re: As suggested by the reviewer, we added the statements as "Based on the findings of the present study, it was estimated that if 10 and 20 mg/day of olanzapine (the usual doses in Japan) would be administered to smokers, about 7 and 14 mg/day, respectively, should be administered to non-smokers in order to obtain the equivalent olanzapine concentrations. These findings imply that the daily doses of olanzapine should be reduced by 7/10 in non-smokers compared with smokers." and

"Based on the findings of the present study, it was estimated that if 200 and 400 mg/day of clozapine (the usual doses in Japan) would be administered to smokers, about 100 and 200 mg/day, respectively, should be administered to non-smokers in order to obtain the equivalent clozapine concentrations. These findings imply that the daily doses of olanzapine should be reduced by 1/2 in non-smokers compared with smokers." to the Discussion section. We also added: "Based on the results of this meta-analysis, we suggest that the doses of olanzapine and clozapine should be reduced by 7/10 and 1/2 in non-smokers compared with smokers in order to obtain an equivalent olanzapine or clozapine concentration. These results are useful as standards to change the doses of olanzapine and clozapine in clinical practice based on the patient's smoking status." to the Conclusion in the revised manuscript.

Authors' responses (Re) to the comments (C) from REVIEWER #2 (Dr. Tore Haslemo)

C1. I have myself submitted data from two different papers to this study, and therefore has some knowledge on how the authors have collected data. One of my previous studies had relevant data on both olanzapine and clozapine, and I submitted data for both of these (clozapine n=33, olanzapine n=40). In the meta analysis the authors only included the clozapine-data. I can not find any information or criteria in the manuscript of this practice, but it might be that there were already "enough" data on olanzapine, and thereby only including clozapine-data.

Re: We are grateful for the reviewer's kind cooperation. In our meta-analyses, there was significant heterogeneity when we included both sets of the reviewer's data. However, as pointed out by the reviewer, we did not describe why we included only one study from your previous studies in the first version of our manuscript. In order to address the reviewer's concern, we included all of the data from the reviewer's studies, and have added the following descriptions in the revised Results section: "Although there was no significant publication bias (p=0.26), significant heterogeneity was observed (I2=50, p=0.04). Since we included two studies by the same authors, we excluded the older study (Haslemo et al., 2006) in the subsequent analyses to reduce the heterogeneity." and in the revised Discussion section: "Additionally, we excluded one study (i.e. Haslemo et al., 2006) in the analyses of olanzapine in order to reduce the heterogeneity. These may have led to a selection bias." as limitations.

C2. Recent large TDM-studies are not mentioned in the manuscript. These are of interest since they allow for correction of several factors by using different statistical approach through multiple regression/linear mixed model. These studies could not be included in the meta-analysis, but could have discussed to relate the effect of smoking to other factors.

Re: Thank you for pointing out this issue. In the previous version of our manuscript, we did not mention the recent large TDM-studies using multiple regression mixed models. Therefore, we now refer to these studies in terms of the influence of the various factors, including the smoking status, on the disposition of olanzapine or clozapine. We have added the following to the revised Discussion section: "Bigos et al., 2008 (n=523) analyzed the population pharmacokinetics of olanzapine, and they determined that sex, smoking and race contribute to the variability in olanzapine clearance. The study also demonstrated that smoking increased the olanzapine clearance by 55%, while also incorporating other confounding factors." and "Li et al., 2012 applied nonlinear mixed-effect modelling to characterize the pharmacokinetics of clozapine in Chinese patients. In the final model, sex and the smoking status were identified as significant covariates for the clearance of clozapine and norclozapine, and smokers had a 1.45-fold higher clearance of clozapine than non-smokers."

C3. The manuscript is lacking some information about the studies that could not be included. The authors state that 42 studies included data on serum concentrations and smoking habits, but 11 olanzapine studies and 18 clozapine studies could not be included. Please state the reason for this, and update Figure 1 with more details on number of studies. Were data from all included studies confirmed by the original authors? Did they not respond?

Re: We requested the data from the authors if the necessary information was not included in the articles (26 authors), however, only five authors responded. This is the main reason why many studies could not be included. According to the reviewer's suggestion, we updated Figure 1, with more details on the number of studies. Because we used other search engines, Scopus and the Cochrane Library, in addition to Medline in order to address another reviewer's comments (C4), we rewrote the sentences highlighted by the reviewer, i.e. "In the present study, we excluded 10 reports (three about olanzapine and seven about clozapine) because the data were not from subjects who had received olanzapine or clozapine for at least a week (Figure 1). When the values were not described or they were given in another scale, we tried to gather information by requesting it from 26 authors, but only five authors responded to our requests. The other nine studies of olanzapine and 12 studies of clozapine could not be included (regarding olanzapine, the mean C/D ratios of olanzapine and its SD were not available for smokers and non-smokers in seven studies; the SD was not given in two studies. Regarding clozapine, the mean C/D ratios of clozapine and its SD were not available for smokers and non-smokers in seven studies; the mean C/D ratios were provided in another scale, i.e. (ng/ml)(mg/kg) in three studies and the SD was not given for two studies)." as the limitations of the study in the revised manuscript.

C4. The authors might comment on the size of this meta-analysis, seen in light of the quality and limited number of included studies (4 and 7 for clozapine and olanzapine respectively). The data material for this meta-analysis is comparable to some of the largest TDM-studies published on this field. Do the authors find it sufficient to search Medline, and not Embase, for the selection of studies for inclusion in this meta-analysis?

Re: As pointed out by the reviewer, the major limitations of the present study were that we used only Medline, while we should also have used the Embase search engine. Although we could not use Embase due to lack of the access authority, we used Scopus and the Cochrane Library as additional search engines in accordance with the reviewer's comments and the comments raised by the Editor (C2). We also added more detailed descriptions regarding the study selection in the revised manuscript. Moreover, we addressed these issues as limitations of the revised manuscript, and added various sentences including this information, i.e. "The major limitations of the present study are that we could not use another search engine, e.g., Embase, due to lack of the access authority, and we could not include the literature published in other languages (not in English) or the data regarding other confounding factors, such as the age, weight, gender, alcohol consumption and how much the subject smoked." to the revised manuscript. Although there were several major limitations stated above, the present study still provides useful information regarding the suggested dose adjustment based on the patients' smoking status.

C5. Cigarette smoking has such an important effect on the pharmacokinetics of these two drugs, that conducting these studies without correcting for other variables might be defended. But in recent years there have been other studies that included several other factors of importance, like co-medication, patient age, gender, ethnicity and genotype (CYP1A2 and UGT1A4). Although the manuscript now clearly state that this is a study of the effect of smoking only, some of these factors could be discussed more in detail or at least be treated more equally.

Re: Thank you for pointing out these issues. According to the reviewer's suggestion, we have now discussed other factors of importance, such as the co-medication, patient age, gender, ethnicity and genotype (e.g., CYP1A2 and UGT1A4) in greater detail in the revised manuscript. Specifically, we added sentences in the Discussion section indicating that: "Many previous clinical studies reported that sex, race, age, co-medication and the genotype could affect the disposition of olanzapine and clozapine. Since estrogen is known to inhibit the activity of CYP1A2, it is not surprising that the clearance of olanzapine and clozapine was reported to be lower in females than in males. Co-medications are also known to affect the disposition of both olanzapine and clozapine. Several drugs, such as ethynilestradiol, fluozetine, fluvoxamine, fluoxetine, fluxoxamine, paroxetine, sertraline, valproate and venlafaxine were reported to increase the blood concentration of olanzapine and/or clozapine through the inhibition of CYP1A2, CYP2D6, CYP3A4 and/or UDP-glucuronyltransferase 1A4. Additionally, carbamazepine, phenobarbital and trimipramine were reported to decrease the blood concentrations of olanzapine and/or clozapine through the induction of CYP1A2 or CYP3A4. Race is known to be associated with variability in the CYP1A2 activity. Bigos et al., 2008 reported that African Americans cleared olanzapine faster than did other races (i.e., Caucasians, Asians and Native Americans). Moreover, many genetic polymorphisms were reported to affect to the disposition of olanzapine and clozapine. A recent review suggested that UGT1A4*3, CYP1A2 rs2472297, FMO3 K158-G308, FMO1*6, FMO1 rs7877 and CYP3A43 rs472660 polymorphisms all influence the olanzapine metabolism. Regarding clozapine, Lee et al., 2012 showed that CYP1A2 rs2069521 and rs2069522 polymorphisms were significantly associated with the C/D ratio of clozapine, and CYP2D6 rs1135840 was associated with the ratio of norclozapine and clozapine."

C6. This manuscript might therefore benefit from removing the additional meta-analysis of factors race and sex, and more clearly sticking to the aim of making practical clinical standards for dose adjustments of clozapine and olanzapine. These findings should be communicated more clearly, also in the abstract, with practical dosing advice rather than solely the observed mean differences.

Re: We entirely agree with the reviewer's comments. As pointed out by the reviewer, the influence of race and sex on the disposition of olanzapine or clozapine was beyond the primary objective of this study. Therefore, we deleted the whole sections regarding these factors in the revised manuscript. Additionally, we added descriptions in the Abstract indicating that: "Based on our findings, we suggest that the doses of olanzapine and clozapine should be reduced by 7/10 and 1/2, respectively, in non-smokers compared with smokers in order to obtain an equivalent olanzapine or clozapine concentration. These results are useful as standards to adjust the doses of olanzapine and clozapine in clinical practice based on the patient's smoking status."

C7. The findings seem valid, but the authors should stick to the primary objective of the study.

Re: We accept the reviewer's comments. The primary objective of the present study was to evaluate the differences in the disposition of olanzapine and clozapine between smokers and non-smokers using a meta-analysis in order to develop standards that can be used to adjust the doses of olanzapine and clozapine used in clinical practice based on the smoking status of the patient. However, as pointed out by the reviewer, the previous version of our manuscript described our results regarding the influence of sex and race. Therefore, in accordance with the reviewer's comments, we deleted all of the results regarding these factors in the revised manuscript.

C8. Abstract should include alternative explanations of the observed results, rather than only mean differences ((ng/ml)/ml/day).

Re: As suggested by the reviewer, we added alternative explanations to the Abstract. Specially, we added the descriptions that: “The C/D ratio was significantly lower in smokers than in non-smokers (p< 0.00001), and the mean difference was -0.75 (ng/mL)/(mg/day) (95% CI -0.89 to -0.61). Therefore, it was estimated that if 10 and 20 mg/day of olanzapine would be administered to smokers, about 7 and 14 mg/day, respectively, should be administered to non-smokers in order to obtain the equivalent olanzapine concentration..” and “The C/D ratio was significantly lower in smokers than in non-smokers (p < 0.00001) and the mean difference was -1.11 (ng/mL)/(mg/day) (95% CI -1.53 to -0.70). Therefore, it was estimated that if 200 and 400 mg/day of clozapine would be administered to smokers, about 100 and 200 mg/day, respectively, should be administered to non-smokers.”

C9. The authors state that 58-88% of patients are smokers, this implies that the vast majority of patients are smokers. Dose correction for smoking might therefore be performed through lowering doses in non-smokers rather than increasing doses in cigarette smokers. Dose reduction (per cent) in non-smokers might be a more suitable practical guideline?

Re: We agree with the reviewer’s comments. As pointed out by the reviewer, the dose correction of olanzapine or clozapine for smoking should be performed by lowering the doses for non-smokers, and this strategy might be a more suitable practical guideline, because 58-88% of patients with schizophrenia are reported to be smokers. In the revised manuscript, we added the following descriptions to the revised Discussion: “Based on the findings of the present study, it was estimated that if 10 and 20 mg/day of olanzapine (the usual doses in Japan) would be administered to smokers, about 7 and 14 mg/day, respectively, should be administered to non-smokers in order to obtain the equivalent olanzapine concentrations. These findings imply that the daily doses of olanzapine should be reduced by 7/10 in non-smokers compared with smokers.” and “Based on the findings of the present study, it was estimated that if 200 and 400 mg/day of clozapine (the usual doses in Japan) would be administered to smokers, about 100 and 200 mg/day, respectively, should be administered to non-smokers in order to obtain the equivalent clozapine concentrations. These findings imply that the daily doses of olanzapine should be reduced by 1/2 in non-smokers compared with smokers.”

C10. Page 8 line 24. “Previous clinical studies reported that smokers had an approximately five-fold lower dose corrected steady-state plasma olanzapine concentration.” Does this represent the current view of the effect of cigarette smoking on olanzapine? This reference (reference 8 Carrillo et al.) is based on a very small study (n=17).

Re: As pointed out by the reviewer, the reference was based on a very small study. In the revised manuscript, we added a reference with a larger sample size, and rewrote the sentence highlighted by the reviewer as follows: “Citrome et al., 2009 (n=380) reported that the olanzapine concentrations were significantly lower in smokers with schizophrenia than in non-smokers. Previous clinical studies with small numbers of patients with schizophrenia reported that smokers had an approximately five-fold lower dose-corrected steady-state plasma olanzapine concentration and a lower decrease in the Brief Psychiatric Rating Scale-total score than non-smokers.”

C11. Page 13 line 46: Since we could not obtain the data regarding the olanzapine disposition during the condition of smoking cessation…. Was it considered part of the aim of this study to determine the effect of smoking cessation on C/D ratios of olanzapine? This kind of data might be available from a few published case reports, but is probably not available in a large scale.

Re: As pointed out by the reviewer, the objective of the present study was to evaluate the differences

in the olanzapine and clozapine disposition between smokers and non-smokers, and there is no data available regarding the influence of smoking cessation on the disposition of these drugs in large-scale studies. Therefore, we deleted the sentences highlighted by the reviewer in the revised manuscript.

C12. Page 14, line 50: Four studies regarding the clozapine disposition met our criteria, all of these were retrospective studies.

Re: According to the reviewer's comments, we rewrote the sentence as: "Four studies regarding the clozapine disposition met our criteria, all of which were retrospective studies."

C13. Page 16, line 12-24 could be removed after stating that all clozapine studies were retrospective (page 14, line 50).

Re: In the revised manuscript, we deleted the sentences highlighted by the reviewer, because all of the clozapine studies were retrospective.

C14. Page 19, line 56: Two and four studies are included for additional meta-analysis of sex and race, and only for olanzapine. Bigos et al. and Citrome et al. might cover and discuss these topics better in their respective papers. Covering these topics are considered outside the scope of this paper, at least when omitting several other important factors.

Re: We entirely agree with the reviewer's comments. In the revised manuscript, we deleted the results of the meta-analysis regarding sex and race, because the analysis was beyond the primary objective of the present study.

C15. Page 19 line 35: Other factors affecting the disposition of clozapine and olanzapine. Co-medication should also be discussed here.

Re: In the revised manuscript, we discussed co-medication in accordance with the reviewer's suggestion. Specifically, we added the following in the revised Discussion section: "Co-medications are also known to affect the disposition of both olanzapine and clozapine. Several drugs, such as ethynilestradiol, fluozetine, fluvoxamine, fluoxetine, fluxoxamine, paroxetine, sertraline, valproate and venlafaxine were reported to increase the blood concentration of olanzapine and/or clozapine through the inhibition of CYP1A2, CYP2D6, CYP3A4 and/or UDP-glucuronyltransferase 1A4. Additionally, carbamazepine, phenobarbital and trimipramine were reported to decrease the blood concentrations of olanzapine and/or clozapine through the induction of CYP1A2 or CYP3A4."

C16. Page 20 line 35: Limitations of the study: The inclusion process should be covered more in detail in this manuscript, both in Figure 1 and in the text. Other patient factors might be covered better: Studies performed in specific patient populations (elderly, women, different diagnostic groups) might lead to increased or reduced impact of smoking. Several large studies in the recent years covered relevant factors like drug interactions, sex, age, race and genetics.

Re: In order to address the reviewer's comments, we added the following sentences in the revised Discussion: "Nevertheless, in the present study, there was insufficient data available to assess the effects of these factors on the disposition of olanzapine or clozapine. Moreover, the influence of smoking on the disposition of olanzapine and clozapine might be different among different patient

populations (e.g., the elderly, females, different diagnostic groups), but we could not conduct a meta-analysis for these populations."

C17. Page 22, line 15: The conclusion of the study does not reflect the aim: "create standards to adjust doses based on smoking status". The conclusion of the study should not request additional research, but rather give practical advice based on the included studies.

Re: In accordance with the reviewer's comments, we added statements such as: "Based on the results of this meta-analysis, we suggest that the doses of olanzapine and clozapine should be reduced by 7/10 and 1/2 in non-smokers compared with smokers in order to obtain an equivalent olanzapine or clozapine concentration. These results are useful as standards to change the doses of olanzapine and clozapine in clinical practice based on the patient's smoking status." to the Conclusion, and we deleted the sentences, "Furthermore, this meta-analysis could not consider the amount of smoking or adherence to olanzapine and clozapine treatment. Therefore, additional research is required to establish an administration plan based on the smoking status of patients" in the revised manuscript.

C18. Figure 1 Flow chart (more details regarding the process of inclusion/exclusion are needed)

Re: In the revised Figure 1, we have described the process of inclusion and exclusion in greater detail in accordance with the reviewer's comment.

C19. Figure 2 Forest plot OLA (n=7) Try to include information from figure 3 and 4 in figure 2

Re: As suggested, we have now incorporated the information included in Figures 3 and 4 in Figure 2. In the revised manuscript, these results are shown in Figure 3.

C20. Figure 3 Forest plot prospective OLA (incorporate information about study type in Figure 2)

Re: In accordance with the reviewer's comment, we incorporated the information from Figure 3 in Figure 2.

C21. Figure 4 Forest plot retrospective OLA (incorporate information about study type in Figure 2)

Re: We have now moved the information included in the previous Figure 4 as Figure 2 in the revised manuscript according to the reviewer's comment.

C22. Figure 5 Forest plot clozapine

Re: Based on the reviewer's comment, we revised the figure legend for Figure 5. In the revised manuscript, the result is shown in Figure 4.

C23. Supplementary figure 1: Funnel plot olanzapine (not stated that Citrome et al. are represented with 3 data points in this figure)

Re: We have revised the figure legend for Supplementary figure 1 in accordance with the reviewer's comment.

C24. Supplementary figure 2: Funnel plot clozapine (n=4) Supplementary figure 3: Race (not sex as stated in figure legends) Supplementary figure 4: Sex (not race as stated in figure legends)

Re: In response to the reviewer's comments, we revised the figure legend for Supplementary figures 2, 3 and 4.

Authors' responses (Re) to the comments (C) from REVIEWER #3 (Dr. Adrian Barnett)

C1. The three results from Citrome are treated as independent in the meta-analysis, but this is unlikely to be true. It would be better to include a random intercept in the mixed effect meta-analysis to acknowledge the correlation in results from the same study. This may widen the overall confidence interval.

Re: As pointed out by the reviewer, we included the three results from Citrome et al., 2009 independently, and, therefore, we should verify the correlation of these results using a random intercept in the mixed effects meta-analysis. When the three results were separately included in the meta-analysis, the weighted differences were not significantly different among the analyses (Supplementary figure 3). However, we could not apply the random intercept in the mixed effects meta-analysis, because we used the Review Manager (RevMan) software program, which lacks this function, in the present analysis. In order to address the reviewer's comments, we added the descriptions stated above to the limitations of the revised manuscript.

C2. Are there any caveats about extending these doses from a meta-analysis to the patient setting?

Re: Based on the results of this study, if 10 and 20 mg/day of olanzapine (the usual doses in Japan) would be administered to smokers, about 7 and 10 mg/day, respectively, should be administered to non-smokers in order to obtain the equivalent olanzapine concentrations. Additionally, if 200 and 400 mg/day of clozapine (the usual doses in Japan) would be administered to smokers, about 100 and 200 mg/day, respectively, should be administered to non-smokers. Therefore, we suggest that the doses of olanzapine and clozapine should be reduced by 7/10 and 1/2, respectively, in non-smokers compared with smokers in order to obtain an equivalent olanzapine or clozapine concentration. These results are useful as standards to adjust the dose of olanzapine and clozapine in clinical practice based on the patient's smoking status. In order to address the reviewer's comment, we added the above information to the Discussion and Conclusion sections in the revised manuscript.

C3. Confounding was not really addressed. Other associations with age and sex are mentioned, but are these confounders of cigarette smoking? Cigarette smoking is often associated with a number of other risky behaviours such as alcohol consumption. Would alcohol consumption effect these drugs?

Re: We entirely agree with the reviewer's comments. The influence of race and sex on the olanzapine or clozapine disposition was beyond the primary objective of this study, although these factors might be associated with the smoking behaviours. Alcohol consumption was reported not to affect the disposition of olanzapine in a previous study (reference 35), whereas there is, to our knowledge, no data available regarding the association between alcohol consumption and the disposition of

clozapine. Nevertheless, in the present study, we could not fully address the other confounders of cigarette smoking, such as alcohol consumption. In accordance with the reviewer's comments and the comment raised by the Editor (C3), we addressed these issues in the revised manuscript by adding information regarding the limitations of our study. Specifically, we added the following sentences: "The major limitations of the present study are that we could not use another search engine, e.g., Embase, due to lack of the access authority, and we could not include the literature published in other languages (not in English) or the data regarding other confounding factors, such as the age, weight, gender, alcohol consumption and how much the subject smoked." to the Discussion section in the revised manuscript.

C4. - Article focus "based on smoking status" no "the"

Re: According to the reviewer's comment, we deleted the word "the" in the revised manuscript.

C5. - Page 7 Line 38 - "shows a" missing a space

Re: In the revised manuscript, we added a space after the word "shows". Thank you for pointing this out.

C6. - Page 9 line 41, the year 1946 seems very early, when were these drugs first available? I note the earliest publication date of included studies is 2008.

Re: Although the earliest publication date among the studies we included is 2008, the first publications regarding olanzapine or clozapine appeared in the 1970s. Therefore, we set the search years as early as possible in the previous version of our manuscript.

C7. - Page 10 line 18, did all subjects need to take the drug for a week? What if only 90% complied with taking the drug for a week or more?

Re: In the present study, we included the studies describing that the subjects took the drug for at least a week, but we could not obtain the information regarding the adherence from all of the studies included. Therefore, we could not divide the subjects taking the drug correctly from those who were not. In the revised manuscript, we rewrote the sentences as: "Although we included the studies that described that the subjects had taken the drug for at least a week, we could not obtain any information regarding the adherence, because none of the studies clearly described this information."

C8. - Page 10 line 44, did any authors not respond to the change of unit request? I see that this information appears later on page 20, but it would be worth flagging here that not all authors responded.

Re: We requested the data from 26 authors if the either the mean C/D ratios or the SD was not described, and only five authors responded. In order to address the reviewer's comments, we rewrote the sentences as "we tried to gather information by requesting it from 26 authors, but only five authors responded to our requests. The other nine studies of olanzapine and 12 studies of clozapine could not be included (regarding olanzapine, the mean C/D ratios of olanzapine and its SD were not available for smokers and non-smokers in seven studies; the SD was not given in two studies. Regarding clozapine, the mean C/D ratios of clozapine and its SD were not available for smokers and non-

smokers in seven studies; the mean C/D ratios were provided in another scale, i.e. (ng/ml)(mg/kg) in three studies and the SD was not given for two studies).” as limitations of the revised manuscript.

C9. - Table 1, is the age for the Citrome et al study the range? (same comment for Weide study in Table 2)

Re: Thank you for pointing out this issue. The age for the Citrome et al. study indicated the range. In the revised manuscript, we added the words “or range” in the revised Table 1.

C10. - Page 13 line 41, 'No significant publication bias'

Re: We rewrote the sentence as “No significant publication bias” in accordance with the reviewer’s comment.

C11. - The planned subgroup analysis should be mentioned in the methods.

Re: We agree with the reviewer’s comment. In the revised manuscript, we added descriptions about the planned subgroup analysis, i.e. “The weighted mean difference was also calculated when the studies were stratified according to the study design, i.e. prospective or retrospective study.”, to the Methods section in the revised manuscript.

C12. - Page 17, spell-out BPRS (and page 18)

Re: We have now spelled out “BPRS”, i.e. Brief Psychiatric Rating Scale, in the revised manuscript.

C13. - Page 17 line 35, 'association' not 'correlation'

Re: We replaced the word “correlation” with “association” in the revised manuscript.

C14. - Page 20, the simple definition of smokers should be mentioned in the methods section and/or introduction

Re: We have now added the descriptions about the simple definition of smokers, i.e. “In this study, the smokers were defined as the subjects who were currently smoking.”, to the Methods section in the revised manuscript.

End of the authors’ responses

In addition to the revisions described above, we have corrected several typos. All corrected portions in the revised manuscript are highlighted.

Thank you again for your pertinent comments, which have greatly helped us to improve our manuscript.

VERSION 2 – REVIEW

REVIEWER	Massimo Carlo Mauri Clinical Psychiatry Clinical Neuropsychopharmacology Unit
REVIEW RETURNED	06-Jan-2014

GENERAL COMMENTS	The article has been corrected as suggested. In my opinion it is now suitable for publication.

REVIEWER	Tore Haslemo Centre for Psychopharmacology, Diakonhjemmet Hospital Oslo, Norway
REVIEW RETURNED	17-Jan-2014

GENERAL COMMENTS	Abstract page 3 line 9: clarity -> clarify Abstracts page 4, line 27: This way of stating the dose reduction in non smokers is easily misinterpreted for olanzapine. Readers could believe either a 0,7 fold reduction, or a reduction to 0,7, i.e. 30% or 70% reduction Please correct throughout the manuscript: "...should be reduced by 30% and 50% respectively." Article summary: Page 6 ...should be reduced by 30% and 50% respectively. Page 11, line 27: Delete: if THE either the PAge 14 line 19 and 22: Identical data for both studies, please check: Number of smokers, gender and age. Page 26 line 15: ...should be reduced by 30% and 50% respectively. Page 28 line 38: English generic names are sufficient when stating potential inhibitors, i.e. fluoxetine and fluvoxamine only. Page 33: line 18: ...should be reduced by 30% and 50% respectively.

VERSION 2 – AUTHOR RESPONSE

Authors' responses (Re) to the comments (C) from REVIEWER #2 (Dr. Tore Haslemo)

C1. Abstract page 3 line 9: clarity -> clarify

Re: Thank you for pointing this out. We have replace the word, "clarity" with "clarify" in the revised manuscript.

C2. Abstracts page 4, line 27: This way of stating the dose reduction in non smokers is easily misinterpreted for olanzapine. Readers could believe either a 0,7 fold reduction, or a reduction to 0,7, i.e. 30% or 70% reduction Please correct throughout the manuscript: "...should be reduced by 30% and 50% respectively."

Re: In response to the reviewer's comments, we revised the sentences highlighted by the reviewer as follows: "…should be reduced by 30% and 50%, respectively".

C3. Article summary: Page 6 ...should be reduced by 30% and 50% respectively.

Re: Based on the reviewer's comment, we revised the sentence as "…should be reduced by 30% and 50%, respectively" in the revised manuscript.

C4. Page 11, line 27: Delete: if THE either the

Re: According to the reviewer's comment, we delete the word "THE" in the revised manuscript.

C5. Page 14 line 19 and 22: Identical data for both studies, please check: Number of smokers, gender and age.

Re: Thank you for pointing out this issue. We have checked the number of smokers, gender and age in these articles and revised the Table 1 in the revised manuscript.

C6. Page 26 line 15: ...should be reduced by 30% and 50% respectively.

Re: Based on the reviewer's comment, we revised the sentence as "…should be reduced by 30%" or "…should be reduced by 50%" in the Discussion section of the revised manuscript.

C7. Page 28 line 38: English generic names are sufficient when stating potential inhibitors, i.e. fluoxetine and fluvoxamine only.

Re: In the revised manuscript, we rewrote the sentence as follow: Several drugs, such as fluoxetine and fluvoxamine, were reported to increase the blood concentration of olanzapine and/or clozapine through the inhibition of CYP1A2, CYP2D6, CYP3A4 and/or UDP-glucuronyltransferase 1A4" in accordance with the reviewer's suggestion.

C8. Page 33: line 18: ...should be reduced by 30% and 50% respectively.

Re: In accordance with the reviewer's comment, we revised the sentence as "…should be reduced by 30% and 50%, respectively" in the revised manuscript.

In addition to the revisions described above, we have corrected several typos. All corrected portions in the revised manuscript are highlighted.

Thank you again for your pertinent comments, which have greatly helped us to improve our manuscript.