METFORMIN IN THE TREATMENT OF NON-ALCOHOLIC FATTY LIVER DISEASE: A META-ANALYSIS

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ABSTRACT
Twenty percent (20%) of the world’s population have Non-Alcoholic Fatty Liver Disease (NAFLD), about 10% of which progresses to Non-Alcoholic Steatohepatitis (NASH) which can lead to cirrhosis and even HCCA. Insulin sensitizing agents such as metformin are theoretically sound and cheap therapeutic options for this disease.

Objectives
To evaluate the effectiveness of diet plus metformin compared to diet alone in improving liver function tests, insulin levels, glucose levels and BMI in patients with NAFLD.

Search Strategy
Cochrane, EMBASE and MEDLINE search was done for randomized controlled trials using free-text terms and MESH words – “Metformin”, “insulin sensitizing agents”, “Non-alcoholic steatohepatitis”, “fatty liver”, “NASH”, “NAFLD”, “Steatohepatitis”, “Non-Alcoholic Fatty Liver Disease”, “Randomized Clinical Trials’” and “Meta-analysis”.

Selection Criteria
Only full-length randomized controlled trial articles which compared metformin and diet in the treatment of NAFLD were included for analysis.

Data Collection and Analysis
All selected studies were evaluated independently for methodological quality by three observers using a validated questionnaire. Inclusion of studies was arrived at by the consensus. After clinical appraisal of each of the included studies, a fixed effect model using odds ratio was used to synthesize the results (Review Manager 4.2).

Results
The literature search yielded two (2) RCT’s with a total study population of 116. ALT significantly decreased with Metformin plus diet treatment (n=116, OR point estimate = 15.78, 95% CI: 5.37, 26.19). There was also a significant decrease in insulin levels in the metformin plus diet group (n=116, OR point estimate = 2.4, 95% CI: 0.88, 3.92). Blood glucose levels showed a trend towards improvement but were not significant (n=116, OR point estimate = 3.68, 95% CI: -1.68, 9.05).

Conclusions
This study shows benefit in the use of Metformin for patients with NAFLD in decreasing ALT and insulin levels. More RCT’s with larger population sizes and endpoints including histopathology have to be done to validate the results of this meta-analysis.

INTRODUCTION
Twenty-five (25%) percent of the general population in the United States have fatty liver, An unpublished data in the Philippines by De Lusong, revealed that 12% of the patients admitted in a tertiary hospitals in a 5 year period diagnosed with fatty liver have Non-Alcoholic Fatty Liver Disease (NAFLD). Ten (10%) percent of the patients with NAFLD progressed to Non-Alcoholic SteatoHepatitis (NASH), while up to one third of these NASH cases can progress to cirrhosis, liver failure and even hepatocellular carcinoma².
NAFLD is now believed to be a widespread liver disease in Western countries and most probably, even in Asian countries. Moreover, it arguably may be the leading cause of cryptogenic cirrhosis. Even with its preponderance, NAFLD remains one of the therapeutic challenges of gastroenterologists and internists alike.

Increase in prevalence of NAFLD is expected because of the increasing rates of obesity and diabetes cases. The proportion for the current world population for obese ranges from 12% to 24% (Philippines 13% men, 15% women) while the proportion of population with diabetes is set to rise in the next 30 years.

Nonalcoholic fatty liver disease (NAFLD) includes a spectrum of liver diseases including fatty liver, steatohepatitis, and even cirrhosis in the absence of alcohol intake known to be injurious to the liver. NAFLD has four histological stages: (1) fatty infiltration of the liver, (2) fatty infiltration plus inflammation, (3) fatty infiltration with ballooning degeneration, and (4) fatty infiltration with lesions similar to alcoholic hepatitis and sinusoidal fibrosis, polymorphonuclear infiltration with or without Mallory hyaline. Nonalcoholic Steatohepatitis or NASH is the name given to the third and fourth stages. It has a benign course in majority of cases, but up to 20% of these patients with NAFLD have NASH.

Recent studies have proposed that obesity and type II diabetes mellitus, which are hyperinsulinemic states with decreased tissue sensitivity to insulin, are the major players in the pathogenesis of NAFLD. This resistance to the effects of insulin seems to be an almost universal underlying feature of NAFLD and NASH and is the rational basis for treatment of NAFLD with weight reducing programs and/or insulin sensitizing agents.

There is currently no established pharmacologic treatment for NAFLD. Weight loss is presently one of the standard treatment measures for NAFLD especially for those who are overweight. Weight loss is usually advocated as an initial treatment; however, the value of this has not been well substantiated. More recently, a study showed that diet and exercise led to a reduction in AST, ALT, serum lipids, and body weight in patients with NAFLD; however, less than 5% of obese patients seem to be able to sustain weight loss.

Several trials using drugs, such as lipid lowering agents like clofibrate and gemfibrozil, ursodeoxycholic acid and some antioxidants, have been used in various studies for the treatment of nonalcoholic fatty liver disease with conflicting results.

In general, resistance to the effects of insulin seems to be an almost universal underlying feature of NAFLD for both Western and Eastern populations. Insulin sensitizing agents such as metformin and thiazolidinediones are theoretically sound therapeutic options which could address this problem. Small, uncontrolled studies have shown biochemical and histological improvement in patients with NAFLD who were treated with pioglitazone or rosiglitazone although hepatotoxicity remains a barrier to this treatment option. Metformin, another insulin sensitizing agent that has likewise been tested in pilot studies in patients with NAFLD has resulted to normal liver tests after treatment. However, in some patients it has shown an improved liver biopsy after 1 year. Oxidant stress, which has also been proposed as a mechanism of liver injury in NAFLD patients, has also been shown to be addressed by metformin. Moreover, metformin is not hepatotoxic as compared to the other insulin sensitizing agents used in recent trials and may be used for the treatment of NAFLD in the long term due to its availability and affordability.

Therefore, combination of studies using metformin in the treatment of NAFLD was sought out as this therapeutic option may prove to be the only drug that could help reverse NAFLD and its dreaded end point, cirrhosis.

Objectives

The aim of this meta-analysis is to determine the efficacy of metformin plus diet restriction amongst patients with NAFLD compared with diet restriction alone. We thus plan to combine all qualified researches, taking into account baseline characteristics, sample sizes and treatments done, in looking for the possibility of a positive outcome in terms of clinical and laboratory parameters for patients treated with metformin.
METHODS
Selection of Studies
The internet was utilized to exhaust almost all possible studies available for our research question. We searched under Google, Cochrane, MEDLINE, and EMBASE data bases for studies restricted to Metformin treatment in humans with NAFLD from January 1966 to June 2005. (See Appendix A)

We also conducted a manual search of references cited in published original articles and review articles restricted to human studies but not limited to English language. Cross references were conducted to check for relevant trials, while consultation with experts and pharmaceutical companies were done to check for published and unpublished randomized controlled trials. Locally published and unpublished studies were also searched for thoroughness.

The Bugianesi study lacked sufficient data for analysis. The author wrote the investigators of the abovementioned trial and were graciously provided with raw data from the study for inclusion into this meta-analysis.

Criteria for considering studies for this review
Types of studies
Randomized Controlled Trials (RCTs) evaluating efficacy of Metformin with diet restriction compared with diet restriction alone in improving liver function tests, insulin levels, glucose levels and BMI in patients with NAFLD.

Types of participants
Patients included 18 years or older and diagnosed with NAFLD based on clinical and laboratory evaluation, including drugs or medications, liver enzyme concentrations, hepatitis markers, anti-hepatitis C virus (anti-HCV)] and autoantibodies, and by histopathology.

Excluded in the study are patients with Hepatitis B or C, alcohol consumption of more than 20g/day, relative or absolute contra-indication for metformin, autoimmune hepatitis, and patients diagnosed with hemochromatosis. Other conditions like history of malignant disease, impaired renal function (serum creatinine > 1.5 mg/dL), heart failure, history of lactic acidosis, severe infection, hypoxic status, serious acute and chronic illnesses, homodynamic instability, age more than 70 years, or the current use of any drugs that may affect the results were also excluded.

Types of interventions
Metformin plus dietary restriction as treatment and dietary restriction alone as control.

Types of outcome measures
Primary outcomes
Mean change from baseline of ALT, AST levels, BMI, insulin levels, and glucose levels.

Secondary outcome
Safety and tolerability of metformin.

Statistical Analysis
Using Revman 4.28 software, weighted mean differences were calculated for each of the two RCT’s and for the combined study population. Test for heterogeneity was also calculated using this software.

Methods of the review
Study Selection
All authors independently searched for potentially relevant trials. Full text articles were retrieved whenever possible. All relevant trials identified were evaluated by three independent reviewers based on the
fulfillment of pre-defined inclusion criteria using a study eligibility form. Any disagreement was resolved by discussion and settled by consensus.

Assessment of Methodological Quality

The quality of the trials eligible for inclusion in the review were assessed by three (3) independent reviewers in terms of generation of allocation sequence, allocation concealment, blinding, intention-to-treat and follow-up using a pre-designed form. For each trial, each factor was assessed as "adequate", "inadequate" or "unclear."

The literature search yielded four (4) clinical trials – with two (2) RCT's that were suitable for inclusion. Both studies were assessed as “adequate” by three (3) independent reviewers.

Data Extraction

Data from each relevant study were extracted independently using a pre-designed data extraction form. Information on study characteristics which included methods used, population studied, interventions performed and outcomes were evaluated. Any disagreement was resolved by discussion with reference to the trial report. Data was then entered into the Review Manager 4.2 software.

Data Analysis

Data was analyzed using the Review Manager ver. 4.2 software. Comparisons of outcomes with binary data were calculated using odds ratio. Presence of heterogeneity among trials was determined by visual inspection of the forest plots and by using the Chi-square test for heterogeneity using a 10% level of statistical significance. When heterogeneity was detected, random effects model was used and potential sources of heterogeneity according to type of intervention, participants and trial setting was explored.

Description of studies

Among the two randomized controlled trials selected, both met the inclusion criteria and were included in the analysis. See Table 1.

Population Studied

Bugianesi et al, included a majority of male patients (63 males/9 females). Uygun et al, also included males as its major population (21 males/13 females). Most of the population were Caucasians. No significant differences were noted between baseline characteristics of patients in both studies except for gender differences.

Description of Intervention

Both trials used Metformin at a dose of 1.7 grams to 2grams per day for 6 months. Intention to treat analysis was done for both trials. All patients had adequate follow up.

Outcome Assessment

Both trials measured clinical, laboratory and histopathologic endpoints. All trials reported adverse events.

Although both studies showed a general reduction in inflammation on liver biopsy, histopathology and liver biopsy, results were not included in this study since the Uygun study was limited to liver biopsies on an optional basis and data of biopsied subjects are still lacking. Moreover, the patients biopsied from the Bugianesi study (also optional) all came from the metformin group of trial which resulted in a significant heterogeneity in the analysis of study groups, as such this was not pursued.

Methodological quality of included studies

Both trials had the same methodological quality (adequate). Both the Uygun study and Bugianesi Study adequately did allocation concealment via sealed envelopes or random numbers. All of them reported blinding of outcome assessment. They all were intention-to-treat analysis and reported number of participants lost to follow-up.

RESULTS
The two (2) RCT’s combined for a total study population of 116. 82 from the Bugianesi study and 34 from the Uygun study. Combination of studies resulted in 44 total patients for the diet arm and 72 patients for the metformin plus diet group. Please see Table 2.

Analysis of these studies with Revman software showed that mean ALT levels in the metformin plus diet treated group significantly decreased from baseline compared to the diet only group (figure 1. n=116, WMD = 15.78, 95% CI: 5.37, 26.19). There was also note of a significant decrease in mean insulin levels from baseline in the metformin plus diet group compared to the diet alone group (figure 3. n=116, WMD = 2.4, 95% CI: 0.88, 3.92). Blood glucose levels showed a trend toward improvement but was not significant (figure 4. n=116, WMD = 3.68, 95% CI: -1.68, 9.05).

Significant heterogeneity was noted for the AST and Glucose levels group. Computations for heterogeneity among the other study groups showed acceptable values. Please see Figures 1 to 5.

Table 1 Characteristics of Included Studies

<table>
<thead>
<tr>
<th>Study ID</th>
<th>Methods</th>
<th>Participants</th>
<th>Interventions</th>
<th>Outcomes</th>
<th>Notes</th>
<th>Allocation Concealment?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uygun et al</td>
<td>RCT</td>
<td>34</td>
<td>Metformin 1.7g/day plus diet VS. diet alone</td>
<td>Decreased ALT/AST, glucose, insulin and BMI levels</td>
<td>Adequate</td>
<td>YES</td>
</tr>
<tr>
<td>Bugianesi et al</td>
<td>RCT</td>
<td>82</td>
<td>Metformin 1.5g to 2g/day plus diet VS. diet alone or Vitamin E</td>
<td>Decreased ALT/AST, glucose, insulin and BMI levels</td>
<td>Adequate</td>
<td>YES</td>
</tr>
</tbody>
</table>

Figure 1 Difference in ALT Levels after 6 Months of Metformin vs Diet

<table>
<thead>
<tr>
<th>Study or Sub-category</th>
<th>Metformin + Diet</th>
<th>Diet</th>
<th>WMD (Fixed)</th>
<th>Weight</th>
<th>YMD (Fixed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>N</td>
<td>Mean (SD)</td>
<td>%</td>
<td>95% CI</td>
</tr>
<tr>
<td>Maricqshi</td>
<td>55</td>
<td>27</td>
<td>45.62 (45.81)</td>
<td>69.62</td>
<td>6.08 [6.89, 25.29]</td>
</tr>
<tr>
<td>Bugianesi</td>
<td>17</td>
<td>17</td>
<td>37.10 (22.23)</td>
<td>16.72</td>
<td>9.70 [3.26, 22.28]</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>72</td>
<td>44</td>
<td></td>
<td>100.00</td>
<td>15.70 [5.37, 26.19]</td>
</tr>
</tbody>
</table>

Test for heterogeneity: Chi² = 1.23, df = 1 (P = 0.27), I² = 19.8%
Test for overall effect: Z = 2.97 (P = 0.003)

Figure 2 Difference in AST Levels after 6 Months of Metformin vs Diet
### Figure 3 Difference in Insulin Levels after 6 Months of Metformin vs Diet

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>Metformin + Diet</th>
<th>Diet</th>
<th>Weight</th>
<th>WMD (fixed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manchesni</td>
<td>55</td>
<td>27</td>
<td>44.06</td>
<td>2.42 [1.58, 3.70]</td>
</tr>
<tr>
<td>Degan</td>
<td>15</td>
<td>17</td>
<td>55.92</td>
<td>15.30 [7.95, 22.65]</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>72</td>
<td>44</td>
<td>100.00</td>
<td>9.62 [4.12, 15.12]</td>
</tr>
</tbody>
</table>

Test for heterogeneity: CH² = 5.20, df = 1 (P = 0.02), I² = 61.8%
Test for overall effect: Z = 3.49 (P = 0.0006)

### Figure 4 Difference in Blood Glucose Levels after 6 Months of Metformin vs Diet

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>Metformin + Diet</th>
<th>Diet</th>
<th>Weight</th>
<th>WMD (fixed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manchesni</td>
<td>55</td>
<td>27</td>
<td>29.79</td>
<td>0.98 [1.80, 3.76]</td>
</tr>
<tr>
<td>Degan</td>
<td>15</td>
<td>17</td>
<td>70.21</td>
<td>3.00 [1.19, 4.61]</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>72</td>
<td>44</td>
<td>100.00</td>
<td>2.48 [0.68, 3.32]</td>
</tr>
</tbody>
</table>

Test for heterogeneity: CH² = 1.42, df = 1 (P = 0.23), I² = 29.8%
Test for overall effect: Z = 3.10 (P = 0.000)

### Figure 5 Difference in BMI after 6 Months of Metformin vs Diet

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>Metformin + Diet</th>
<th>Diet</th>
<th>Weight</th>
<th>WMD (fixed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manchesni</td>
<td>55</td>
<td>25</td>
<td>72.46</td>
<td>2.65 [1.70, 3.60]</td>
</tr>
<tr>
<td>Degan</td>
<td>15</td>
<td>17</td>
<td>27.66</td>
<td>6.52 [1.93, 16.73]</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>72</td>
<td>42</td>
<td>100.00</td>
<td>3.68 [1.68, 9.06]</td>
</tr>
</tbody>
</table>

Test for heterogeneity: CH² = 0.41, df = 1 (P = 0.53), I² = 46%
Test for overall effect: Z = 1.35 (P = 0.18)
Adverse Events

Both trials reported no stoppage of medication due to side effects from metformin. Only a few patients from the Uygun study complained of bloatedness. These did not require cessation of intake of metformin.

DISCUSSION

NAFLD remains to be one of the most elusive diseases of the century. Despite efforts aimed at improving early detection and prevention, most patients are still seen at the advanced stages of this illness. Current treatment options are still inadequate to treat all stages of the disease. Other treatment modalities mentioned beforehand are still in the experimental stage and currently have no place in clinical practice. As such, the present situation of NAFLD therapeutics is still unsatisfactory. Our pursuit for other treatment options are thus enhanced and justified based on the abovementioned problems.

The effectiveness of metformin as an insulin sensitizer has already been proven and is currently one of the main treatment options in the management of diabetes. Metformin increases insulin-mediated glucose utilization in peripheral tissues and has an anti-lipolytic effect that lowers serum free fatty acid concentrations. Significant decreases in alanine aminotransferase and insulin levels in the metformin plus diet treated group compared to the diet treated group alone may translate into clinical benefits. Previous studies on thiazolidinediones have noted that improvement in liver enzymes as well as insulin levels correlated well with histopathologic improvement on liver biopsies of patients with NAFLD. This study increases evidence for metformin in that 6 month treatment of NAFLD with metformin and dietary support significantly reduces ALT and insulin levels compared to dietary support alone. This was in contrast to the recent study by Nair et al. 2004 where there was only transient improvement in transaminases. ALT improvement is somewhat similar to figures reported by studies using thiazolidinediones. Metformin is less expensive than most other treatment modalities for NAFLD and is such affordable to the affected population. Most studies with chronic or prolonged use of Metformin have shown very few or little side effects and thus can be used safely for long periods of time. This was also reflected in this study with no patient withdrawing from metformin for intolerance or side effects.

Drawbacks of this study include the small population size which may not be an adequate representative sample. Although studies included in this analysis are few, they were still adequate for inclusion in the analysis. Moreover, there is paucity of trials using metformin compared to diet in the treatment of NAFLD. Most of the studies included, concentrated on patients with steatohepatitis and fibrosis. This may mean that
metformin may be better suited for patients with liver biopsy. Further studies to verify these statements will have to be done in the near future.

In summary, results show that metformin seems to decrease ALT and insulin levels which may reverse the inflammatory activity in the liver, as well as improve insulin and glucose parameters thus further decreasing lipolysis and fatty acid production and damage to the liver. Moreover, it is well tolerated and seems to have negligible side effects when used for 6 months.

CONCLUSION
Implications for practice
The study showed advantages in the use of Metformin for patients with NAFLD coupled with dietary support. Six months of metformin for patients with NAFLD is well tolerated and seems to lead to improvement of ALT and insulin levels. The trials, however, numbered only two, and included very small sample sizes, as such the authors could not therefore conclude with certainty if metformin is to be included as one of the considerations for pharmacologic therapy for similar patients in treating NAFLD.

Implications for research
Due to the promising results of this study, the authors strongly urge that more RCT’s with larger population sizes and adequate randomization be done to verify these results. Moreover, studies should also include histopathologic parameters as end points, with reversal of inflammation to normal or at least delay of progression to fibrosis to further strengthen and validate the results of this meta-analysis.

Potential conflict of interest
None known.

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APPENDIX A
SEARCH STRATEGY
The search method was as follows:

For NAFLD:
- From the “Pub MED” site, in the search field, the free text search word “fatty liver”, “NASH”, “NAFLD”, “Non-Alcoholic Steatohepatitis”, “Steatohepatitis”, and “Non-Alcoholic Fatty Liver Disease”, was entered.
- The MESH term “fatty liver” was entered into the search box of PUBMED.
- Combining the results of the above search terms with “OR” yielded 15318 matches.

For Metformin:
- Search terms, “metformin”, “insulin-sensitizing agents” were entered as free text into the search field.
- The MESH term “Metformin” was also entered.
- Combining result of both searches above with “OR” yielded 3251 matches.
Intersecting together the results for both NASH and Metformin with “AND” resulted in 43 matches. Removing non-randomized controlled trials from these results further limited our results to 4 clinical trials, of which, 2 were Randomized Controlled Trials (RCT’s).

Of the matches retrieved from the above search, a separate search filtering for meta-analysis yielded zero articles.