Metformin and beta cell function in insulin-treated patients with type 2 diabetes: a randomized placebo-controlled 4.3 year trial

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**Abstract**

In this trial, 390 insulin treated patients with type 2 diabetes were randomized to either placebo or metformin. Fasting levels of glucose, insulin and C peptide were determined at baseline, after four months and yearly after for four years to assess fasting estimates of beta cell function. The primary endpoint was the fasting C peptide to glucose ratio (FCPGR) and secondary measures were the disposition index (DI) and the fasting C peptide (FCP). We analyzed the results with a general linear mixed model. Baseline FCPGR was 5.27 (95% CI 4.83 - 5.71). Compared to placebo, FCPGR increased in the metformin group with 1.48 (95%CI 1.09 - 1.87, p<0.001). The DI showed comparable results with a treatment effect of 1.50 (95%CI 1.17 - 1.83; p<0.001). FCP also increased in the metformin group but did not reach statistical significance vs placebo (0.034 nmol, 95%CI, -0.005 - 0.072; p=0.085). Treatment with metformin versus placebo, added to insulin in patients with type 2 diabetes, improves long term estimates of beta cell function in the fasting state.
**Introduction**

Metformin is a key drug in the treatment of type 2 diabetes. In our ‘Hyperinsulinaemia: the Outcome of its Metabolic Effects (HOME) Study’ we showed that metformin vs placebo improved glycemic control and decreased insulin requirements in insulin-treated patients with advanced type 2 diabetes (1).

In this respect, metformin is generally regarded as an insulin sensitizer. Whether metformin also may improve beta-cell function is not clear. In the present analysis of the HOME study, we studied the effects of metformin vs placebo on estimates of fasting beta-cell function. In addition, we quantified the durability of metformin’s effect on these estimates over a period of 4.3 years.

**Methods**

**Design and patients**

In the HOME trial, 390 insulin-treated patients with advanced type 2 diabetes were randomly allocated to either metformin 850 mg (up to thrice daily if tolerated) or matching identical looking placebo through a computer program. Most patients (345) were already using monotherapy insulin, either twice daily premixed NPH/regular insulin (Novomix) or NPH insulin in the evening combined with prandial regular insulin. The remaining 45 patients used a combination of metformin and insulin and stopped metformin three months before randomisation.

All participants provided written informed consent and the study was approved by the medical ethical committees of the three participating non-academic hospitals. Study visits were at baseline, at one month and three monthly after with a follow up of 4.3 years.

**Measures**

Blood samples for this analysis of the HOME trial were drawn at baseline and after 4, 17, 30, 43, and 52 months, and stored at -80°C until analysis. C-peptide plasma samples were available for 363 patients at baseline and at one or more follow up visits (93%), and for 259 patients at their final visit (66%) and was performed with a solid-phase, chemiluminescent enzyme immunoassay (Immulite 2000, DPC). Serum insulin was measured by electrochemiluminescence immunoassay (Modular E170, Roche Diagnostics). Coefficients of variation are provided in the appendix. Because patients used human basal insulin (Insulatard, Novo Nordisk) there was full cross-reactivity between endogenous insulin and exogenous insulin in this assay.
The primary estimate of beta cell function was the fasting C peptide to fasting plasma glucose ratio (FCPGR). To normalise our ratio based on SI units, we used an arbitrary constant of 100 for convenience resulting in the unitless formula FCPGR = 100 * FCP/FPG. As secondary measures we used the fasting C-peptide (FCP) and the disposition index (DI), defined as the FCPGR adjusted for insulin sensitivity (IS) resulting in the unitless formula DI = FCPGR * IS^0.2. Insulin sensitivity was calculated from fasting plasma glucose (FPG) and fasting plasma insulin (FPI) using the unitless formula IS = 1000/FPG*FPI. For further details we refer to the statistical appendix.

**Statistical analysis**

We used all measurements to assess the effects of metformin vs placebo during the total period of follow-up. In order to quantify the overall treatment effect over time, we used a linear mixed model simultaneously assessing the significance of the main time effect, main metformin treatment effect, and interaction of metformin treatment effect with time (detail in the statistical appendix).

In addition we did a mediation analysis to assess the indirect (mediating) effect of HbA1c in the change of FCPGR. For this purpose, we added HbA1c as a covariate in the model and evaluated the mediating HbA1c effect by the product of the effects (metformin→HbA1c) and (HbA1c→FCPGR), and confidence interval calculated by bootstrapping (detail in the statistical appendix).

**Results**

Figure 1 shows the time course of HbA1c, beta cell function and insulin sensitivity during all visits. Table 1 shows the results of the mixed linear model in which, for each variable, the baseline value, time effect, treatment effect and time-treatment interaction are shown. Treatment effect in the model is defined as the constant post-baseline change in the metformin group versus placebo, expressed as an absolute change. In addition this change is described as a relative change compared to baseline.
Table 1. Mixed model fixed effects parameter estimates

<table>
<thead>
<tr>
<th></th>
<th>FCPGR Disposition index</th>
<th>C-peptide (nmol/l)</th>
<th>Insulin sensitivity</th>
<th>HbA1c (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>5.27 [4.83; 5.71]</td>
<td>4.73 [4.36; 5.10]</td>
<td>0.50 [0.46; 0.54]</td>
<td>0.90 [0.74; 1.07]</td>
</tr>
<tr>
<td>Treatment effect(^{(1)})</td>
<td>1.48* [1.09; 1.87]</td>
<td>1.50* [1.17; 1.83]</td>
<td>0.03 [-0.00; 0.07]</td>
<td>0.33* [0.03; 0.63]</td>
</tr>
<tr>
<td>Time effect</td>
<td>-0.00 [-0.09; 0.08]</td>
<td>-0.08* [-0.15; -0.01]</td>
<td>0.00 [-0.01; 0.01]</td>
<td>0.00 [-0.05; 0.08]</td>
</tr>
<tr>
<td>Time-treatment interaction</td>
<td>-0.12 [-0.26; 0.01]</td>
<td>-0.09 [-0.20; 0.02]</td>
<td>0.01 [-0.01; 0.02]</td>
<td>-0.03 [-0.14; 0.08]</td>
</tr>
</tbody>
</table>

Data are estimates [95% CI]; Time effect and time-treatment interaction are expressed as change per year; *p<0.05
\(^{(1)}\) Constant treatment effect from the first post-baseline visit until the end of the study.

Compared with placebo group, FCPGR increased in the metformin group. Mixed model results showed a constant treatment effect from the first post baseline visit until the end of the trial of 1.48 (95%CI 1.09 - 1.87, p<0.001), no change in time (0.00/year, 95%CI -0.001 - +0.001; p=0.92) and no time-treatment interaction (-0.01/year, 95%CI -0.021 -0.00; p=0.058). Relative to baseline, the treatment effect was 28% (95%CI 23-33).

DI showed results comparable to those with the FCPGR: a small decrease in the placebo group and an increase in the metformin group. Mixed model results confirmed a significant decrease in time of -0.01/year (95%CI-0.01 - -0.001; p=0.023) for placebo group, a constant treatment effect during the whole post baseline period of 1.50 (95%CI 1.17 - 1.83; p<0.001), and no time-treatment interaction (-0.01, 95%CI -0.02 -0.00; p=0.128). Relative to baseline, the treatment effect was 32% (95%CI 27-36).

FCP (nmol/l) decreased in the placebo group and increased in the metformin group. Mixed model results showed a non-significant treatment effect (0.034 nmol, (95%CI, -0.005 - 0.072; p=0.085), no time effect (0.00 nmol/l/year, 95% CI, -0.00 - 0.00; p=0.61), and no time-treatment interaction (0.00 nmol/l/year, 95%CI, -0.00 - 0.00; p=0.26). Relative to baseline, the treatment effect was 7% (95%CI -1;14).

HbA1c (%) increased in the placebo group and decreased in the metformin group. Mixed model results confirmed a significant change in time of 0.07 %/year in placebo (95%CI 0.04 - 0.10; p<0.001), a treatment effect of -0.93 % ( (95%CI -1.06 - -0.80; p<0.001) from the first post-baseline visit until the end of the trial, and a time-treatment interaction of 0.011 %/year (95%CI 0.008 - 0.015; p<0.001).

We assessed the indirect (mediating) effect of HbA1c in FCPGR improvement in adding in the model HbA1c as covariate. In comparing with the initial model, the mediating effect of HbA1c (Metformin→ HbA1c→FCPGR) on the overall effect (Metformin→FCPGR) was
Figure 1 Time course of HbA1c, FCPGR and IS, data are means with standard error of the mean.
small (.53, 95%CI .45, .73), accounting for a small proportion of the variance (36.1%; 95% CI, 25.4-49.5).

Insulin sensitivity increased in the metformin group and decreased in the placebo group. Mixed model results showed a treatment effect of 0.33 (95%CI 0.03-0.63; p=0.031), no significant change in time for placebo (0.00/year, 95%CI -0.00 - 0.01; p=0.69), and no time-treatment interaction (-0.00/year, 95%CI -0.01 - 0.01; p=0.612). Relative to baseline, the treatment effect was 36% (95%CI 3-69).

**Discussion**

The present study shows that metformin added to insulin, improves fasting based estimates of beta cell function durably in comparison with placebo. These effects were for the greatest part (64%) independent of changes in glycemic control. Also adjusting for insulin sensitivity by calculating the disposition index, did not alter the results.

Depending on the estimate used, the increase in beta cell function was 28% (95%CI 23-33) for FCPGR and 32% (95%CI 27-36) for DI. Insulin sensitivity, assessed by a HOMA-derived fasting index, also improved by 36% (95%CI: 3-69).

A C-peptide with a concurrent glucose level of > 8 mmol/l might be considered a non-fasting value (2). This did apply to our population with a mean fasting glucose at baseline of 10 mmol/l. To adjust for this hyperglycemic stimulus, we chose the FCPGR as our primary endpoint.

Meier et al. (3), showed a good correlation of FCPGR with human pancreatic beta cell mass in a small group of patients that underwent pancreatic surgery. Okuna(4) showed in a much bigger population of type 2 diabetes patients, that FCPGR strongly correlates with accepted measures as the HOMA-B (r=0.79) and hyperglycaemic clamp (iAUCins/gluc90, r = 0.721).

Because beta cell function depends on prevailing insulin sensitivity, we adjusted for insulin sensitivity by calculating the disposition index. The use of exogenous insulin may confound the assessment of insulin sensitivity, unless a steady state has been achieved without fast changes in glucose transport (5). In our study fasting insulin levels were drawn in the morning during which a standard condition (a certain steady state) of intermediate acting NPH insulin levels had been achieved without fast changes in insulin driven glucose transport.
Further, a valid DI should incorporate independent estimates of beta cell function and insulin sensitivity (6). Because we used C-peptide-based data for our beta cell estimate and insulin-based data for our insulin sensitivity estimate, we avoided intrinsically interdependent estimates. To assess this independency, we did an additional correlation analysis that showed a weak correlation ($R = 0.29$ (95% CI 0.19-0.38) for C-peptide and insulin.

Although the improvements in beta cell function and IS were maintained during the 4.3 year follow-up period, there was no time-treatment interaction for both beta cell function and IS, indicating that the improvement constitutes an immediate treatment effect without additional change over time relative to placebo.

There are two major trials (7,8) which evaluated long-term metabolic changes related to beta cell function in metformin users, as compared to other treatment modalities: the UK Prospective Diabetes Study (UKPDS) and the A Diabetes Outcome Progression Trial (ADOPT). Both trials also observed modest improvement of beta cell function in metformin users. However, in the UKPDS long term follow up of beta cell function is difficult to interpret because of the slowly rising FPG during the study and its influence on the HOMA-B estimate which was used (9).

In the ADOPT, apart from HOMA-B, the oral glucose tolerance test (OGTT) was used as an estimate of beta cell function. It was shown that metformin during 4 years of follow up improved beta cell function as compared to glyburide, although less pronounced than with rosiglitazone and with a much smaller effect size (2.5%) than in our study (28%).

This may be partially explained by differences between the study populations. Patients included in the ADOPT study were newly diagnosed patients, while in the HOME trial, patients with advanced diabetes on insulin therapy were included. Advanced type 2 diabetes is known to have more beta cell failure than new-onset type 2 diabetes, and may have more potential for improvement (provided beta cell damage is partially reversible). Moreover our placebo controlled design allows a comparison to placebo instead of the comparator based design of the ADOPT.

Although metformin has no direct short term insulin secretory effects in beta cells of normal glucose tolerant individuals (10,11), multiple mechanisms may explain its beta cell enhancing effect in type 2 diabetes patients. Apart from decreased glucotoxicity, an improved incretin secretion (12,13) and a reduced lipotoxicity (14) may be involved in the action of metformin to improve beta cell function.
Our study is limited by studying the effects of metformin on estimates of beta cell function and insulin sensitivity in the fasting state. Effects of metformin through the incretin system may also improve prandial beta cell function, which we did not study in the present study.

In conclusion, the present study shows that metformin results in long term improvement in fasting estimates of beta cell function in addition to an improvement in insulin sensitivity, contributing to a durably improved glycemic control in insulin-treated patients, even in advanced type 2 diabetes.

**Article information**

WMT and PL primary analyzed the data. WMT and AK drafted the manuscript, CS and AK reviewed the manuscript. AK, CS and PL are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. We thank all members of the HOME study group and all patients for their contribution. This part of the HOME Trial was supported by grants from Merck, Sharpe, & Dohme and Novo Nordisk. The sponsors had no role in the design and conduct of the study; in the collection, analysis, and interpretation of the data; or in the preparation, review, or approval of the manuscript. All the authors declare that there are no relevant conflicts of interest to disclose.
References


Supplemental appendix

Methods

For the insulin assay the coefficients of variation were analysed by the CLSI-EP10 protocol in which total coefficients of variation are computed from the within-run SD and inter-run SD by means of an Analysis of Variance (ANOVA) experiment. The total coefficients of variation 1.6; 1.8 and 2.4% and the intra-assay coefficients of variation were 1.0; 0.5 and 0.9% at concentration levels of 71; 406 and 720 pmol/l, respectively.

For the C-peptide assay the total coefficients of variation (CV) were 17.7; 6.2 and 4.1% and the intra-assay coefficients of variation were 14.4; 4.0 and 3.2% at concentration levels of 0.3; 1.2 and 2.0 nmol/l, respectively.

Mixed model

To assess the effects of metformin vs placebo on the various variables, we used a general linear mixed model simultaneously assessing :

a) the constant main effect of metformin vs placebo during post baseline period;
b) the time effect, as the change over time for the placebo group;
c) the time * treatment interaction to measure the variation of the metformin effect over time;
d) the intercept or constant value at baseline for the placebo group.

By defining $Y_{ik}$ as the value of each studied endpoint for the patient $i$ at time $k$, and treatment by $\theta$ (0=placebo, 1=metformin), this model can be written as:

$$Y_{ik} \sim \beta_0 (1+ N(0,\sigma_i)) + \beta_1 (1+ N(0,\sigma_s))t_{ik} + \beta_2 \theta_i + \beta_3 \theta_i t_{ik} + E_{ik} \tag{1}$$

The $\beta$’s are coefficients of the four effects to be estimated and $E_{ik}$ is the residual of the model. $\beta_0$ is the average score of the studied endpoint at M0 for the placebo group and is constituted of a fixed part ($\beta_0$), but may vary among patients according to a normal distribution $N(0,\sigma)$ of mean=0 and with a standard deviation of the intercept $\sigma_i$; $\beta_1$ is the average trend across time (slope) for the placebo group, but may vary among patients with a standard deviation of the slope $\sigma_s$; $\beta_2$ measures the mean difference between the two studied drugs during the whole post-baseline period; $\beta_3$ interaction term measures the mean difference between the two drugs across the trial span.

In this model, treatment $\theta$ was considered as changing variable with $\theta=0$ at baseline and $\theta=1$ for all the other periods. Time $t_{ik}$ had values of 0, 4, 17, 30, 43 and 52 months. As the four studied values are correlated, we conjectured the same model for the six measured endpoints.
Insulin sensitivity
Because we analysed fasting insulin levels and because metformin is assumed to mainly influence hepatic glucose production, we used an estimate that primarily reflects hepatic insulin sensitivity. Matsuda (1) showed that the inverse of the product of fasting plasma glucose and fasting insulin levels, closely correlates with hepatic insulin sensitivity. This is mathematically identical to the original HOMA formula, in which the constant is arbitrary. We chose a constant of 1000, to adjust for the use of SI units and the hyperinsulinemia in our study population. This results in the formula IS = 1000/FPG*FPI which we used unitless.

Disposition index
The FCPGR was calculated by the formula 100 * FCP/FPG. Because the physiological hyperbolic relationship between insulin secretion and insulin sensitivity may not be present in the pathophysiology of type 2 diabetes, we analyzed their actual baseline relationship in our study population. We performed a log-transformed linear regression in which this relation was best described by the formula FCPGR = 2.81 * IS^{-0.2}. The resulting DI is calculated with the formula DI = FCPGR * IS^{0.2} (2)

Mediation analysis
The mediation effect of HbA_{1c} of the effect of Metformin treatment on FCGR was assessed by a univariate mediation effect calculated as follows: for every visit I+1, compared with visit I, let be
a) the global effect of Treatment (trt) on FCGR modeled as: FCGR(i+1) = k0 + k1*FCGR(i) + c*trt;

b) the effect of HbA_{1c} on FCGR modeled as HbA_{1c} (i+1) = k0 + k1* HbA_{1c} (i) + a*trt;

c) the effect of HbA_{1c} and Trt on FCGR modeled as FCGR(i+1) = k0 + k1*FCGR(i) + b* HbA_{1c} (i) + d*trt;

the mediation effect of the combination Trt\rightarrow HbA_{1c} and HbA_{1c} \rightarrow FCGR is calculated as the ratio a*b/c

the point and interval estimates were calculated by a bootstrap process of 1000 repetitions
References

1 Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care September 1999 22:9 1462-1470
