Changes in glucose and A1c prior to the diagnosis of diabetes.
A 10 year follow-up of the Hoorn Study

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Submitted
ABSTRACT

Objective
To study changes in fasting glucose, postload glucose and A1c over a ten-year period in persons who did and did not develop diabetes in the Hoorn Study.

Research design and methods
For the analysis we used data of 565 non-diabetic subjects between 50-75 years of age who had an OGTT and A1c determination in 1989, 1996 and 2000 (The Hoorn Study). Diabetes was diagnosed at follow-up based on fasting glucose ≥ 7.0 mmol/l and/or postload glucose ≥ 11.1 mmol/l and/or A1c ≥ 6.5%. Linear mixed models, using follow-up times of 6.3 years (1989-1996) and 10 years (1989-2000), were used to compare the time course of glycemic measures in participants who did and did not develop diabetes.

Results
Of the 565 participants, 147 developed diabetes within 10 years. Developmental patterns of fasting and postload glucose were equal in the first 6.3 years of follow-up in participants who did and did not develop diabetes after 10 years of follow-up. In the last 3.7 years of follow-up, a rapid increase of fasting and postload glucose was seen in those with incident diabetes in 2000. The difference in the increase in A1c between those who did and did not develop diabetes in 2000 was already significant after 6.3 years and further increased using a 10 year follow-up period.

Conclusions
A1c levels start to increase up to 10 years before the diagnosis of diabetes, followed by a rapid increase in both A1c levels and glucose levels a few years before the diagnosis of diabetes.
BACKGROUND

Type 2 diabetes has since long been defined by deviation of glucose levels in the fasting and/or the postload state (1). Elevated glucose levels below the cut-off point for diagnosing diabetes, known as intermediate hyperglycemia, have been shown to predict the development of diabetes (2). Moreover, a recent study investigated the trend in glucose levels before the diagnosis of diabetes and showed increases in glucose concentrations, even below the cut-off point for intermediate hyperglycemia, 3-6 years before the diagnosis of diabetes (3).

Recently, an International Expert Committee reported on the use of A1c for the diagnosis of diabetes (4). A1c is an indirect measure of mean blood glucose over the previous months with more recent glucose levels (in the previous month) explaining 50% of the A1c level (5). As a result of the standardization of the A1c assay and practical advantages of A1c measurement over glucose (6), the use of an A1c level ≥ 6.5% for the diagnosis of diabetes was proposed (4). This advice was officially adapted by the American Diabetes Association in their guidelines for the diagnosis of diabetes in 2010, in which it is advised to combine high levels of glucose and A1c in the diagnosis of diabetes (7). Indeed, many epidemiological studies showed increasing risk for diabetes as well as cardiovascular disease with increasing A1c levels (8;9) and a strong relationship between glucose and A1c in patients with diabetes (10;11). However, in people without diabetes, the association between glucose and A1c has been found to be less strong compared to people with diabetes (12). It has been demonstrated earlier that other factors which are not related to glucose metabolism, for example genetic factors, aging, differences in erythrocyte lifespan and racial differences are determinants of A1c (13-16). Thus A1c might be less sensitive in reflecting glycemia in the normal range. Furthermore, it is unknown whether early changes in glucose levels prior to the diagnosis of diabetes, as shown in previous studies (3), are accompanied
by changes in A1c. To evaluate this, we compared changes in fasting glucose, postload glucose and A1c levels over a 10-year period in participants of the Hoorn Study who did and did not develop diabetes.

METHODS

Study population
The Hoorn Study is a population-based cohort study of diabetes, which started with 2484 men and women, aged 50-75 at baseline in 1989. Follow-up examinations were performed in 1996-98 (further referred to as 1996) and 2000. In the 1996 follow-up examination, all the surviving participants (n=2086) were invited for a second examination to which 1513 participated. In 2000, all subjects with fasting glucose ≥ 6.1 mmol/L or postload glucose levels ≥ 7.8 mmol/L in 1996 (n=176) and a sample of those with normal glucose metabolism (n=705) were invited. Of these 1074 persons, 647 (60%) agreed to participate. For the present analysis, we excluded patients with diabetes in 1989 (n=73). Diabetes was defined according to the 2010 criteria of the American Diabetes Association: elevation of glucose (fasting glucose ≥ 7.0 mmol/L, postload glucose levels ≥ 11.1 mmol/L or A1c (≥ 6.5 %) (7) or use of glucose lowering medication. We furthermore excluded 9 persons with missing data on both A1c and OGTT in 1996 or 2000. So finally, 565 subjects who were free of diabetes at the baseline visit were included in the present analysis. All participants signed informed consent and the study was approved by the medical ethics committee of the VU University Medical Center.

Laboratory analysis
All blood samples were analyzed at the clinical chemistry laboratory of the VU University Medical Center. A1c levels were determined by ion-exchange high performance liquid chromatography (HPLC) on a modular diabetes monitoring system (Bio-Rad, Veenendaal, the Netherlands) in 1989 and 1996.
In 2000, a slightly different ion-exchange HPLC procedure was used, based on separation on a Mono-S column (Pharmacia, Uppsala, Sweden) using previously described methodology (17). An internal validation report by our laboratory, in which both procedures to determine A1c were compared in 200 patients, provided a linear regression model of the agreement between the two methods: A1c value Pharmacia = (1.039 * A1c value Bio-Rad) – 0.020.

Fasting and postload glucose concentrations were assessed by means of a glucose dehydrogenase method at the first and second examination (Merck, Darmstadt, Germany) and by a hexokinase method (Roche, Mannheim, Germany) at the third examination. Triglycerides, total and HDL-cholesterol were determined from fasting blood samples by enzymatic techniques (Roche, Mannheim, Germany) at all time points.

**Statistical analysis**

Firstly, the regression function mentioned in the paragraph ‘laboratory analysis’ was used to transform Bio-Rad A1c values into Pharmacia A1c values and, as a result, increase the comparability of A1c values over time. Then, subjects were categorized into three groups: no diabetes at baseline or during follow-up, incident diabetes in 1996 and incident diabetes in 2000. Baseline differences between the three groups were tested with Student’s t-test for continuous data and chi-square tests for proportions. Next, the proportion of participants with impaired fasting glucose (IFG, fasting glucose 6.1-7.0 mmol/l), impaired glucose tolerance (IGT, postload glucose 7.8-11.1 mmol/l) and/or elevated A1c (A1c levels 6.0-6.5%) at every examination were calculated.

Linear mixed models with random intercept were used to compare the time course of glycemic measures (fasting glucose, postload glucose and A1c) in all three groups. Linear mixed models (also referred to as random coefficient analysis) account for the correlation between repeated measurements and allow for the use of time-dependent and time-independent variables (18). A random intercept allows the baseline value to vary between subjects, while
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changes over time are fixed. Since we expected a non-linear association between diabetes status and glycemic measures over time, we categorized follow-up time in the model as the follow-up duration since the baseline examination (i.e. 6.3 and 10.0 years, respectively). Fasting glucose, postload glucose and A1c were standardized before inclusion in the models, reporting the standard deviation (SD) as unit of change. The models produce estimates for the effects of diabetes status after 6.3 or 10 years, time, and interaction between time and diabetes status. The estimate for the effect of diabetes status reflects the baseline difference per SD of the glycemic measure between those who did versus those who did not develop diabetes after 6.3 or 10 years. The estimate for the effect of time represents the change per SD of the glycemic measure over time among those without diabetes. The estimate for the interaction term group*follow-up time reflects the additional effect of an increase or decrease per SD of the glycemic measure for those who did versus those who did not develop diabetes after 6.3 or 10 years. All analysis were performed in SPSS 15.0.0 (SPSS Inc., Chicago, IL). P<0.05 was considered statistical significant.

RESULTS

Of the 565 study participants, 147 persons (26%) developed type 2 diabetes within 10 years of follow-up, of whom 99 developed diabetes between 1989 and 1996 and 48 between 1996 and 2000. The diagnosis of 73 of these 147 persons (49.6%) was by elevation of fasting and/or postload glucose levels exclusively and 43 persons (29.5%) had diabetes by elevated A1c exclusively.

Table 1 shows the baseline characteristics, stratified for diabetes status during follow-up. Compared to participants who did not develop diabetes, patients with incident diabetes in 1996 or 2000 already had elevated levels of fasting glucose, postload glucose and A1c in 1989. In addition, they had an adverse cardiovascular risk profile at baseline, characterized by for example low HDL cholesterol levels and high mean waist circumference. The proportions of IFG,
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IGT and elevated A1c in 1989 and 1996 are presented for participants with and without diabetes in 1996 and 2000 in the bottom rows of Table 1. The baseline prevalences of IFG, IGT and/or elevated levels of A1c in those with incident diabetes in 2000 were 20.8%, 29.2% and 29.2% respectively. Between 1989 and 1996, these prevalences further increased toward 56.3% IFG, 45.8% IGT and 60.4% elevated A1c.


<table>
<thead>
<tr>
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<tbody>
<tr>
<td>N</td>
<td>418</td>
<td>99</td>
<td>48</td>
</tr>
<tr>
<td>Age (years)</td>
<td>59.0 (6.2)</td>
<td>60.8 (7.0)*</td>
<td>63.0 (6.7)*</td>
</tr>
<tr>
<td>Gender (% male)</td>
<td>48.1</td>
<td>51.5</td>
<td>47.9</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>5.3 (0.5)</td>
<td>5.8 (0.6)*</td>
<td>5.7 (0.5)*</td>
</tr>
<tr>
<td>Postload glucose (mmol/L)</td>
<td>5.2 (1.5)</td>
<td>6.7 (2.1)*</td>
<td>6.7 (2.0)*</td>
</tr>
<tr>
<td>A1c (%)</td>
<td>5.4 (0.5)</td>
<td>5.8 (0.5)*</td>
<td>5.7 (0.5)*</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>6.5 (1.2)</td>
<td>6.8 (1.4)</td>
<td>6.7 (0.9)</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.4 (0.4)</td>
<td>1.3 (0.4)</td>
<td>1.2 (0.4)</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.4 (0.7)</td>
<td>1.7 (1.6)*</td>
<td>1.9 (1.0)*</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>129.0 (17.2)</td>
<td>138.6 (19.3)*</td>
<td>139.6 (22.4)*</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>80.9 (9.8)</td>
<td>84.5 (10.8)*</td>
<td>83.1 (10.1)</td>
</tr>
<tr>
<td>Smoking status (% smoking at baseline)</td>
<td>24.9</td>
<td>34.7</td>
<td>35.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.8 (3.0)</td>
<td>27.1 (3.2)*</td>
<td>27.1 (3.6)*</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>88.3 (10.1)</td>
<td>93.8 (9.8)*</td>
<td>94.4 (11.0)*</td>
</tr>
<tr>
<td>IFG 1989 (%)</td>
<td>5.3</td>
<td>35.4</td>
<td>20.8</td>
</tr>
<tr>
<td>IFG 1996 (%)</td>
<td>24.9</td>
<td>-</td>
<td>56.3</td>
</tr>
<tr>
<td>IFG 2000 (%)</td>
<td>18.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IGT 1989 (%)</td>
<td>6.5</td>
<td>32.2</td>
<td>29.2</td>
</tr>
<tr>
<td>IGT 1996 (%)</td>
<td>14.1</td>
<td>-</td>
<td>45.8</td>
</tr>
<tr>
<td>IGT 2000 (%)</td>
<td>23.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Elevated A1c 1989 (%)</td>
<td>10.0</td>
<td>38.4</td>
<td>29.2</td>
</tr>
<tr>
<td>Elevated A1c 1996 (%)</td>
<td>14.4</td>
<td>-</td>
<td>60.4</td>
</tr>
<tr>
<td>Elevated A1c 2000 (%)</td>
<td>28.2</td>
<td>-</td>
<td>-</td>
</tr>
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</table>

Data are presented as mean (SD) or percentage. IFG = impaired fasting glucose (6.1-7.0 mmol/l); IGT = impaired glucose tolerance (7.8-11.1 mmol/l), elevated A1c = A1c 6.0-6.5 %.

*p < 0.05 as compared to the group without diabetes
Time-dependent changes in fasting and postload glucose and A1c in the three groups are presented in Figure 1. In participants with incident diabetes in 1996, mean fasting glucose, A1c and postload glucose increased from 1989 to 1996. After the diagnosis in 1996, all three glycemic measures stabilized towards 2000. Mean levels of fasting glucose and postload glucose in participants with incident diabetes in 2000 increased gradually between 1989 and 1996, but with a comparable slope as in those without diabetes. In contrast, A1c levels already show a slight increase in future diabetes patients between 1989 and 1996 compared to those without diabetes. Between 1996 and 2000, rapid increases in fasting glucose, and especially A1c and postload glucose are seen in those who developed diabetes in 2000. The interpretations of Figure 1 are confirmed by the results of the linear mixed models, presented in Table 2. As can be seen from the baseline difference, persons who developed diabetes already had higher fasting and postload glucose levels and A1c as compared to those who did not develop diabetes. The interaction terms between diabetes status and time are significant for all glycemic measures in the analysis using patient with incident diabetes in 1996 (Table 2, top rows, last two columns), indicating that there is a difference in the change of glycemic measures over time in those without diabetes as compared to those with incident diabetes in 1996. This difference in change is comparable using a 6.3 year or a 10 year interval.
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Figure 1.
Changes in fasting and postload glucose and A1c in those with incident diabetes in 1996 (top lines, N=99), those with incident diabetes in 2000 (dotted lines, N=48) and those who did not develop diabetes during 10 years of follow-up (bottom lines, N=418). Data are presented as the mean and 95% confidence intervals at all time points.
Figure 2.
Changes in fasting glucose, postload glucose and A1c in those with incident diabetes in 2000 based on diabetic HbA1c levels only (N=17, dotted lines) or diabetic glucose levels only (N=20, black lines), compared those who did not develop diabetes during 10 years of follow-up (N=418, bottom lines). Data are presented as the mean and 95% confidence intervals at all time points.
The interaction term of diabetes status and time in the analysis in which participants with incident diabetes in 2000 were used, is small and non-significant for fasting glucose (0.02 (-0.24-0.29) SD) and for postload glucose (0.03 (-0.20-0.27) SD) in the first 6.3 years of follow-up. This implicates that in the first 6.3 years of follow-up, there is no difference in the change of fasting glucose and postload glucose in those without diabetes and those with incident diabetes in 2000. Using a 10 year interval, the group*time interaction in relation to fasting and postload glucose increased and became significant (0.70 (0.43-0.96) SD and 0.78 (0.54-1.03) SD respectively). In contrast to glucose, the interaction between diabetes status and time in relation to A1c was already significant in the first 6.3 years (0.34 (0.09-0.58) SD), which further increased to 0.84 (0.59-1.09) SD using 10 years of follow-up. To study whether the above mentioned patterns were consistent in groups with diabetes based on high glucose levels solely or high A1c levels solely, we separated the diabetes patients in 2000 into three groups: diabetes based on diabetic glucose levels only (N=20), diabetes based on diabetic A1c levels only (N=17) and diabetes based on both high glucose and high A1c (N=11). In Figure 2, changes in A1c and glucose in the first two groups (diabetes based on diabetic glucose levels only and diabetes based on diabetic A1c levels only) are compared to changes in those without diabetes. The figure shows that in those with an A1c-based diagnosis, mean A1c levels are higher at all time point compared to those with a glucose-based diagnosis, whereas glucose levels are higher at all time point in those with a glucose-based diagnosis. Furthermore, A1c levels tend to increase in patients with a glucose-based diagnosis of diabetes, while levels of fasting glucose and postload glucose stabilized in those with an A1c-based diagnosis of diabetes.
Table 2. Linear mixed models showing the baseline and time-dependent differences in hyperglycaemia measures in those without diabetes compared to those with incident diabetes in 1996 or incident diabetes in 2000.

<table>
<thead>
<tr>
<th>Incident diabetes in 1996</th>
<th>Baseline group difference a</th>
<th>Change over time b</th>
<th>Group*time interaction c</th>
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<tr>
<td></td>
<td>SD</td>
<td>Estimate</td>
<td>(95% CI)</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>0.857</td>
<td>0.63 (0.46-0.81) a</td>
<td>0.54 (0.45-0.62) d</td>
</tr>
<tr>
<td>Postload glucose (mmol/L)</td>
<td>2.362</td>
<td>0.63 (0.44-0.81) a</td>
<td>0.26 (0.19-0.34) d</td>
</tr>
<tr>
<td>A1c (%)</td>
<td>0.553</td>
<td>0.78 (0.59-0.96) a</td>
<td>0.18 (0.10-0.26) d</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Incident diabetes in 2000</th>
<th>Baseline group difference e</th>
<th>Change over time f</th>
<th>Group*time interaction g</th>
</tr>
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<td>A1c (%)</td>
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<td>0.47 (0.20-0.70) a</td>
<td>0.18 (0.10-0.26) d</td>
</tr>
</tbody>
</table>

Estimates are expressed per standard deviation change in hyperglycaemia measure. Diabetes diagnosis: fasting glucose ≥ 7.0 mmol/L, postload glucose levels ≥ 11.1 mmol/L or A1c ≥ 6.5% (7) or use of glucose lowering medication.

a Represents the baseline difference between persons with incident diabetes in 1996 versus those without diabetes
b Represents the time-dependent changes in those without diabetes during the first follow-up period (estimate 1, after 6.3 years) and the second follow-up period (estimate 2, after 10 years)
c Represents the time-dependent changes in risk factor attributable to diabetes status in 1996 of the first follow-up period (estimate 1, after 6.3 years) and the second follow-up period (estimate 2, after 10 years)
d P<0.001
e Represents the baseline difference between persons with incident diabetes in 2000 versus those without diabetes
f Represents the time-dependent changes in those without diabetes during the first follow-up period (estimate 1, after 6.3 years) and the second follow-up period (estimate 2, after 10 years)
g Represents the time-dependent changes in risk factor attributable to diabetes status in 2000 of the first follow-up period (estimate 1, after 6.3 years) and the second follow-up period (estimate 2, after 10 years)
DISCUSSION

In summary, patients who develop diabetes already have elevated levels of glucose and A1c 10 years before diagnosis, including high prevalences of IFG/IGT. Levels of glucose and A1c increase over time, with significantly higher levels at all time points in those who developed diabetes compared to those who did not. When comparing the time course of the increase in glucose and A1c before diagnosis of diabetes, a rapid increase in fasting, postload glucose and A1c shortly before diagnosis can be observed. Moreover, in contrast to glucose, A1c levels of diabetes patients already start to slightly increase 10 year before diagnosis.

Time trends in glucose levels before the diagnosis of diabetes have been described previously. In the Whitehall II Study, screening of fasting and postload glucose during a median follow-up of 9.7 years was reported. Results showed that glucose levels increased annually with 0.02-0.8 mmol/l and rapidly 2-3 years before the diagnosis of diabetes (3). Mason et al. showed in a study in 55 diabetic Pima Indians a same pattern for postload glucose, with a rapid increase on average 4.5 years before diagnosis (19). This is confirmed by our results in which we found a steady increase followed by a rapid increase of fasting glucose and especially postload glucose shortly before diagnosis. Based on our results and the results from previous studies, the diagnosis of diabetes might be preceded by different stages of glucose elevation, insulin resistance and insulin secretion, resulting in periods with slow increases in glucose levels, followed by a period shortly before diagnosis in which a rapid increase is the result of a discordance between insulin secretion and insulin resistance (3;20;21).

Earlier studies investigated the predictive value of A1c for incident diabetes (8), but our study is, to our best knowledge, the first to describe changes in A1c over a 10 year time period in persons who did and did not develop diabetes.
We showed that A1c levels increased rapidly shortly before diagnosis, which is comparable to the developmental pattern of fasting and postload glucose. It is often suggested that A1c reflects average glucose levels (10). Our results confirm that glucose and A1c are comparable in their developmental pattern shortly before diagnosis, which might implicate that A1c indeed reflects average glucose levels. This is confirmed by the subgroup analysis, in which we observed increases in A1c-levels in patients with a glucose-based diabetes diagnosis, underlining the role of glycation in the formation of HbA1c. On the other hand, the subgroup analysis also showed that in those with an A1c-based diagnosis, glucose levels are stable. A1c is known to be influenced by several other factors, like differences in erythrocyte lifespan and genetic factors (13,15,22). It could be speculated that genetic predisposition to glycation or physiological processes leading to changes in erythrocyte environment up to 10 years before diagnosis are among the explanations of the earlier increase in A1c as compared to glucose and the differences in developmental patterns of glucose and A1c in patients with a glucose-based or A1c-based diagnosis. However, the precision of the measurements of glucose and A1c might also play a role. The analytical and biological variation, expressed as the within-person covariant of variation, of fasting glucose, postload glucose and A1c are 5-7%, 15-18% and 3-4% respectively (23). The high variation of glucose as compared to A1c might have prevented us from detecting a moderate increase in glucose, similar to the increase in A1c, up to 10 years before the diagnosis of diabetes.

Some limitations of our study should be addressed. Firstly, due to the selection procedure used in the follow-up measurement in 2000, loss to follow-up occurred. To study if this affected our results, we compared levels of glucose and HbA1c in 1989 and 1996 in those who did (N=647) and those who did not (N=870) participate in 2000, stratified according to glucose status in 1996. No significant differences were found in the population who participated in 2000 compared to the non-participants in the increase in glucose and HbA1c
between 1989 and 1996. Secondly, it would have strengthened our results if we would have had the availability of more than 3 measurements over 10 years of follow-up. However, the patterns in glucose observed in our study are in line with other studies (3;19) which used a comparable time frame, suggesting that we were able to detect proper patterns using these 3 measurements in this population. Thirdly, the power of our study was not sufficient to extensively study subgroups of patients with diabetes based on high glucose levels solely or high A1c levels solely. More research is needed to investigate if changes in glucose and A1c differ between groups with a different diabetes diagnosis. Last, we were able to adjust for the different A1c assays used, which increased the precision of our results presented. However, we cannot exclude that subtle differences in the laboratory procedures during the 10 years of follow-up have had an effect on the results.

To conclude, we have found rapid increases in fasting glucose, postload glucose and A1c about 4 years before the diagnosis of diabetes. In addition, A1c levels already start to slightly increase 10 years before diagnosis. More research is needed to further unravel the (pathophysiological) mechanisms behind differences in trajectories of glucose and A1c before the diagnosis of diabetes and to study differences in developmental patterns in people with a glucose-based or A1c-based diabetes diagnosis.

Author’s contribution
E.R. researched data, wrote manuscript; M.A. researched data, reviewed/edited manuscript; T.T. reviewed/edited manuscript; G.N. initiated data collection, reviewed/edited manuscript; C.D.A.S. initiated data collection, reviewed/edited manuscript; J.M.D. initiated data collection, reviewed/edited manuscript.

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The authors have no relevant conflict of interest to disclose.
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REFERENCE LIST


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