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**Mechanisms underlying glycemic deterioration in type 2 diabetes: An IMI DIRECT study**

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6

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96    **Abstract**

97    *Objective*

98    We investigated the processes underlying glycemic deterioration in type 2 diabetes (T2D).

99    *Research Design and Methods*

100   732 recently diagnosed T2D patients from the IMI-DIRECT study were extensively phenotyped

101   over three years, including measures of insulin sensitivity (OGIS), β-cell glucose sensitivity (GS)

102   and insulin clearance (CLIm) from mixed meal tests, liver enzymes, lipid profiles, and baseline

103   regional fat from MRI. The associations between the longitudinal metabolic patterns and $HbA_{1c}$

104   deterioration, adjusted for changes in BMI and in diabetes medications, were assessed via stepwise

105   multivariable linear and logistic regression.

106   *Results*

107   Faster $HbA_{1c}$ progression was independently associated with faster deterioration of OGIS and GS,

108   and increasing CLIm; visceral or liver fat, HDL-cholesterol and triglycerides had further

109   independent, though weaker, roles ($R^2$=0.38). A subgroup of patients with a markedly higher

110   progression rate (fast progressors) was clearly distinguishable considering these variables only

111   (discrimination capacity from AUROC=0.94). The proportion of fast progressors was reduced from

112   56% to 8-10% in subgroups in which only one trait among OGIS, GS and CLIm was relatively

113   stable (odds ratios 0.07 to 0.09). T2D polygenic risk score and baseline pancreatic fat, GLP-1,

114   glucagon, diet, and physical activity did not show an independent role.

115   *Conclusions*

116   Deteriorating insulin sensitivity and β-cell function, increasing insulin clearance, high visceral or

117   liver fat, and worsening of the lipid profile are the crucial factors mediating glycemic deterioration

118    of T2D patients in the initial phase of the disease. Stabilization of a single trait among insulin

119    sensitivity, β-cell function, and insulin clearance may be relevant to prevent progression.

120 Maintaining glucose levels within appropriate limits in patients with type 2 diabetes (T2D) is a

121 crucial factor to prevent complications. Effective strategies to slow glycemic progression can be

122 supported by understanding the processes underlying deterioration of glucose control.

123 Few studies have assessed $HbA_{1c}$ trajectories and the possible determinants of glycemic

124 deterioration. An established finding is that β-cell function decline is an important factor (1,2),

125 while contradictory conclusions were drawn for insulin sensitivity (1,3–7). Whether heterogeneous

126 patterns between patients exist in β-cell function and insulin sensitivity decline has not been

127 clarified, an important question for patient stratification and personalized medicine. Other

128 limitations of previous analyses include the incomplete characterization of the metabolic parameters

129 affecting glucose homeostasis (derived using fasting data only (2,4)), the restricted set of traits

130 investigated together, and the lack of potentially relevant measures such as ectopic fat, insulin

131 clearance, or lifestyle. No study has assessed the relationships between the longitudinal trajectories

132 of $HbA_{1c}$ and those of the other metabolic traits.

133 In this analysis, we have used data from the cohort of recently diagnosed and extensively

134 phenotyped T2D patients of the DIRECT study (8,9) to elucidate the processes underlying glycemic

135 deterioration. Specific features of the DIRECT study are the detailed assessment of the glucose

136 homeostasis parameters, and patients all being in the initial phase of the disease. We determined the

137 patterns over a 3-year period of $HbA_{1c}$, β-cell function, insulin sensitivity and other relevant

138 laboratory, clinical and functional parameters, and assessed their relevance in the deterioration of

139 glucose control.

140 **Research Design and Methods**

141 *Subjects and protocol*

142 The IMI-DIRECT (Innovative Medicines Initiative - Diabetes Research on Patient Stratification)

143 project is a multicenter prospective study on northern European adults (8,9) (ClinicalTrials.gov

144     identifier NCT03814915). The present analysis considers the DIRECT cohort of recently diagnosed

145     T2D patients, who were recruited according to the following criteria: white race, T2D diagnosis

146     according to the American Diabetes Association 2011 criteria (10) not less than 6 months and not

147     more than 24 months before baseline examination, previous treatment via lifestyle measures with or

148     without metformin therapy, age between 35 and 74 years, BMI between 20 and 50 kg/m$^2$, estimated

149     glomerular filtration rate >50 ml/min, and HbA$_{1c}$ concentration <7.64 % (60.0 mmol/mol) within

150     the previous 3 months. Participants were studied at baseline (month 0) and at months 9, 18 and 36.

151     Subjects with HbA$_{1c}$ available at least in two visits were included in this analysis (*N*=750).

152     All participants provided written informed consent and the study protocol was approved by the

153     regional research ethics review boards. The research conformed to the ethical principles for medical

154     research involving human participants outlined in the declaration of Helsinki.

155     *Collected data*

156     Anthropometric data, HbA$_{1c}$, blood lipids and liver enzymes were collected at all visits. A 27-month

157     HbA$_{1c}$ sample was collected in 39 patients. A standardized mixed meal test (8) (MMTT) was

158     performed at months 0, 18 and 36 to calculate indices of insulin sensitivity (in fasting conditions,

159     QUICKI (11), and post-MMTT, OGIS (12)), *β*-cell function (13) (glucose sensitivity, GS, and rate

160     sensitivity), and insulin clearance (in fasting conditions, and post-MMTT, CLIm). From the

161     baseline visit we collected glucagon, proinsulin and glucagon-like peptide 1 (GLP-1), measures of

162     regional fat from MRI (8) (available in 561 participants), of physical activity from accelerometer

163     (8), and of self-reported 24-hour nutrient intake (8), and we computed the fatty liver index (FLI)

164     (14) and a T2D polygenic risk score (PRS) (15). The whole set of traits considered in this study is

165     described in detail in the Supplemental Material (DATA, METHODS, and Table S2).

166     *Assessment of progression rates*

167      We computed the progression rates for $HbA_{1c}$ and several traits available at follow up

168      (Supplemental Table S4). Each trajectory was described with a conditional linear mixed-effect

169      model (16), in which the longitudinal component of the data was described as a proportional

170      function of time, with normally distributed slopes describing individual progression rates. $HbA_{1c}$

171      progression was adjusted for changes in BMI and diabetes medications, which were recorded at all

172      visits (as dosage and start and end of treatment). The adjustments were assumed to be 1)

173      proportional to BMI; 2) linearly related to the metformin dose, expressed as percentage of a

174      maximal dose of 3 grams; 3) linearly related to the cumulative dose for the other antidiabetic drugs

175      (insulin excluded), expressed as sum of the percentages of the maximum dose of each drug; 4)

176      constant under insulin treatment. A proportional effect of delay in $HbA_{1c}$ assay, i.e. of the difference

177      between the time of measurement and the time of sample collection, was also introduced.

178      Medications were considered to be effective if taken at least 30 days before HbA1c measurement.

179      OGIS and QUICKI trajectories were adjusted for changes in BMI. Further details about the

180      conditional linear mixed-effect models are provided in the Supplemental Material (METHODS).

181      *Statistical analysis*

182      Results are presented for participants (*N*=732) with GAD <11 U/ml and islet antigen-2 antibodies

183      (IA-2) <7.5 U/ml, to exclude other possible forms of diabetes (17). Distributions are described as

184      mean ± standard deviation. Pairwise associations between continuous variables were assessed using

185      the Spearman correlation coefficient; differences between groups were assessed using the Wilcoxon

186      signed rank test (for two groups) and Kruskal-Wallis test (for three or more groups).

187      We used stepwise multivariable linear regression to determine the set of variables, as baseline

188      values (Table S2) and progression rates (Table S4), independently associated with the $HbA_{1c}$

189      progression rate, with adjustment for center, sex and age. For baseline variables, both

190      untransformed and transformed values were considered; transformations were logarithmic, or logit

191      when variables where constrained within an interval. The independent variables were included in

192 the regression model when their effects had $p<0.05$ and produced an increment in the adjusted $R^2$

193 value. Two stepwise analyses were performed: one on all participants, excluding MRI variables

194 from the analysis, and one on the subset of participants with MRI data, including this data in the

195 analysis. Standardized coefficients were computed per standard deviation of the underlying data

196 distribution.

197 Since the distribution of $HbA_{1c}$ progression rates was skewed to the right with a group of patients

198 with high values, we split the subjects into *average* and *fast* progressors according to a progression

199 rate threshold (see Results). We used multivariable logistic regression to assess the odds ratios of

200 average *vs* fast progression, using the independent variables identified in the multiple linear

201 regression analysis of $HbA_{1c}$ progression. The logistic analysis provided values for AUROC,

202 sensitivity, specificity and accuracy, to be used as measures of the discrimination capacity of the

203 investigated independent variables over fast *vs* average progressors. These parameters must not be

204 interpreted as measures of predictive capacity.

205 *Role of the funding source*

206 The funders had no role in study design, in collection, analysis, and interpretation of data, in writing

207 of the report, or in the decision to submit the paper for publication. The corresponding author had

208 full access to all data and had final responsibility for the decision to submit for publication.

209 **Results**

210 *Subjects' baseline characteristics*

211 At baseline, the participants had age of 62±8 years, were moderately obese (30.4±4.9 kg/m$^2$ BMI),

212 and had $HbA_{1c}$ of 6.41±0.53 % (46.5±5.8 mmol/mol) and fasting glucose of 7.1±1.4 mmol/l. (Table

213 S2). 34% of the subjects were treated with metformin at baseline, the rest was treatment naïve.

214 *Progression rates of $HbA_{1c}$ and other traits*

215    The individual $HbA_{1c}$ progression rates (Supplemental Figure S1), adjusted for changes in BMI and

216    in diabetes medications, were on average only slightly positive and mostly distributed close to their

217    median (median, first and ninth deciles were 0.041, -0.038 and 0.185 %/year (0.45, -0.41 and 2.02

218    mmol mol$^{-1}$ year$^{-1}$), respectively). However, the distribution showed a heavy right tail with values

219    up to 0.897 %/year (9.8 mmol mol$^{-1}$ year$^{-1}$). The adjustment of progression rates for BMI changes

220    implied a standardized coefficient for the BMI effect of 0.37.

221    All the other investigated traits had a mean progression rate per year smaller, in absolute value, than

222    5% of the corresponding baseline average (see Table S5 for details). On average, waist

223    circumference, but not BMI, increased very slightly. Insulin sensitivity (as OGIS) and most of the

224    β-cell function parameters decreased. Fasting, but not post-meal, insulin clearance decreased. Total

225    cholesterol did not change, while its fractions showed opposite changes, with HDL increasing and

226    LDL decreasing; TG increased. Creatinine and ALT did not change, while AST and AST/ALT

227    increased.

228    Several pairwise associations were observed between $HbA_{1c}$ progression rate and laboratory,

229    clinical, and functional parameters (Supplemental Figure S2). In particular, $HbA_{1c}$ progression rate

230    was clearly associated ($p<0.01$) with some baseline traits (positively with BMI, waist

231    circumference, triglycerides, glucagon, liver and visceral fat; inversely with age, HDL, insulin

232    sensitivity, and β-cell function) and some progression rates (positively with those of triglycerides

233    and liver enzymes; inversely with those of insulin sensitivity, β-cell function, AST/ALT ratio, and

234    HDL).

235    Several pairwise associations were also observed between the progression rates of the investigated

236    traits (Figure S2, panel B). GS and OGIS progression rates were independent of one another despite

237    $HbA_{1c}$ progression rate being associated with both of them.

238    *Variables associated with $HbA_{1c}$ progression rate: multivariable linear analysis*

239     In multivariable linear analysis of $HbA_{1c}$ progression rate in all patients, the baseline values and the

240     progression rates of several traits provided an independent contribution (adjusted $R^2$ 0.38; Figure 1,

241     panel A). Faster $HbA_{1c}$ progression was independently associated with lower baseline values and

242     faster deterioration of insulin sensitivity (as OGIS) and β-cell function (mostly as glucose

243     sensitivity, GS), with higher baseline values of MMTT insulin clearance, CLIm, and with its

244     increase (all p-values <0.001). Faster $HbA_{1c}$ progression was also independently associated with

245     lower baseline HDL ($p<0.05$) or its slower increase ($p<0.001$), with a quicker increase of TG

246     ($p<0.001$), as well as with higher baseline values of BMI ($p<0.01$) and lower baseline values of

247     $HbA_{1c}$ ($p<0.001$). The variables with strongest effects were the baseline OGIS value and the

248     progression rates of OGIS, GS and CLIm (standardized coefficients, in absolute value, between

249     0.24 and 0.57).

250     In multivariable analysis of the subset of patients with baseline MRI measurements (adjusted $R^2$

251     0.40; Figure 1, panel B), baseline visceral fat was positively and independently correlated with

252     $HbA_{1c}$ progression rate; moreover, female sex and younger age independently predicted faster

253     $HbA_{1c}$ progression. The role of the other key metabolic parameters, OGIS, GS and CLIm, remained

254     similar. Replacing visceral fat with liver fat produced similar results (standardized coefficient equal

255     to 0.15 for visceral fat, to 0.11 for liver fat); when both visceral and liver fat were included in the

256     model, the latter was not independently associated with $HbA_{1c}$ progression.

257     No independent effects were detected for smoking status, family history, T2D polygenic risk score,

258     baseline values of diet, physical activity, pancreatic fat, GLP-1 (total and intact at fasting, total at 60

259     min), glucagon, and 60-min proinsulin, baseline values and progression rates of AST and ALT.

260     Further details on the multivariable linear analysis are reported in the Supplemental Material

261     (RESULTS).

262     *Variables associated with $HbA_{1c}$ progression rate: multivariable logistic analysis*

263    The threshold selected to separate the heavy right tail of the distribution of $HbA_{1c}$ progression rates

264    was 0.255 %/year (2.79 mmol mol$^{-1}$ year$^{-1}$). This threshold split the subjects into average

265    progressors ($N$=699), with a progression rate of 0.044±0.076 %/year (0.48±0.83 mmol mol$^{-1}$ year$^{-1}$),

266    and fast progressors ($N$=33), with a ~10-fold mean progression rate (0.460±0.185 %/year,

267    5.03±2.02 mmol mol$^{-1}$ year$^{-1}$) (Figure 2).

268    We found that the trajectories of most variables independently affecting $HbA_{1c}$ progression as from

269    the linear analysis were clearly different ($p$<0.001) in the two groups (Figure 2): in fast progressors,

270    OGIS and GS strongly declined and TG and CLIm markedly increased. At baseline, fast

271    progressors had lower OGIS ($p$<0.05), CLIm ($p$<0.01) and HDL ($p$<0.001), and higher BMI

272    ($p$<0.01).

273    Logistic analysis substantially confirmed the results of linear regression (Figure 1), with half the

274    investigated variables still contributing ($p$<0.05) to distinguish average and fast progressors (Figure

275    3): fast $HbA_{1c}$ progression independently associated with stronger deterioration and a lower

276    baseline value of OGIS and GS, CLIm increase, and HDL reduction. The discrimination capacity of

277    the logistic model, computed as AUROC, was 0.94 (95% CI between 0.86 and 0.98).

278    Similar outcomes were obtained using lower $HbA_{1c}$ progression rate thresholds, which resulted in

279    larger numbers of patients classified as fast progressors (Supplemental Material - RESULTS,

280    Figures S1 and S3).

281    At baseline, the percentage of patients treated with metformin were not different between fast

282    progressors (39.4% [24.7-56.3%, 95% CI]) and average progressors (33.9% [30.5-37.5%], $p$ =

283    0.64). At the last visit, the percentage of patients treated with any diabetes medication was

284    somewhat higher in fast progressors, as expected ($p$ = 0.048, details provided in the Supplemental

285    Material - RESULTS). Only 7 average progressors were on insulin at the last visit.

286    *Impact of stable OGIS, GS or CLIm on proportion of fast $HbA_{1c}$ progressors*

287   Because HbA$_{1c}$ progression was associated with worsening of three main factors, OGIS, GS and

288   CLIm, we have evaluated the possible importance of maintaining one of these key traits relatively

289   stable in order to avoid fast progression. For this purpose, we considered each trait as deteriorating

290   if its progression rate fell within its worst tertile (the bottom tertile for OGIS and GS, the top one

291   for CLIm), and as stable if it fell in the other two tertiles. We examined the subgroups of patients in

292   which none or only one of these key traits was relatively stable (Table 1).

293   We found that the proportion of fast progressors was 56% in the patient subgroup where GS, OGIS

294   and CLIm were all deteriorating, and decreased to 8-10% in the subgroups where a single trait,

295   either GS, OGIS or CLIm, was stable. All proportions were different from 0 at 90% confidence

296   level, stressing that fast progression did not imply quick changes for each of the three considered

297   traits. All differences in proportions (one stable trait *vs* none) had $p<0.001$, and were associated to

298   odds ratio for fast *vs* average progression below 0.1 (Table 1); thus, relatively stable progression

299   rate of one single trait among GS, OGIS and CLIm was strongly associated to reduced glycemic

300   deterioration.

301   **Conclusions**

302   Leveraging on the detailed participant characterization of the DIRECT study, we have been able to

303   elucidate the processes underlying glycemic deterioration in T2D patients in the initial phase of the

304   disease. We found that HbA$_{1c}$ deterioration was independently associated with 1) a decrease in

305   insulin sensitivity; 2) a decrease in β-cell function (primarily β-cell glucose sensitivity); 3) an

306   increase in insulin clearance; 4) lower values of insulin sensitivity and glucose sensitivity and

307   higher values of insulin clearance at baseline. Further variables independently associated with faster

308   HbA$_{1c}$ progression were declining HDL, increasing TG and high baseline visceral or liver fat.

309   The variables identified by multivariable linear analysis also explained the rapid HbA$_{1c}$

310   deterioration detected in a subset of patients (identified as fast progressors), the strongest predicting

311   variables of the multivariable linear model being significant also with logistic analysis. Clear

312     differences were evident between fast and average HbA$_{1c}$ progressors (Figure 2), consistent with the

313     associations derived from the multivariable linear analysis. The high discrimination capacity of the

314     logistic analysis suggests that the selected variables capture the most relevant pathophysiological

315     factors underlying glycemic deterioration.

316     The independent associations with HbA$_{1c}$ progression of several variables, in particular the

317     progression rates of insulin sensitivity, β-cell function and insulin clearance, and the existence of

318     fast HbA$_{1c}$ progressors with relatively stable conditions for any of these three traits (Table 1),

319     indicates 1) that the processes of glycemic deterioration are heterogeneous in this population of

320     T2D patients; 2) that fast progression does not imply quick deterioration of a specific trait, e.g.

321     insulin sensitivity or β-cell function.

322     The dichotomous analysis shows that the odds for fast *vs* average progression are substantially

323     reduced when either glucose sensitivity, insulin sensitivity or insulin clearance is relatively stable.

324     Although these findings do not demonstrate causality, they suggest that preventing either high

325     degradation rates of glucose sensitivity or insulin sensitivity, or high increase rates of insulin

326     clearance, may be an effective strategy to slow down glycemic deterioration in the initial phase of

327     the disease. This reemphasizes the importance of lifestyle interventions aiming at controlling insulin

328     resistance, as preventing deterioration of the other traits currently appears more difficult.

329     This study also shows that insulin resistance plays a major role in glycemic deterioration in these

330     T2D patients. In particular, we show associations of glycemic deterioration with baseline insulin

331     sensitivity and its longitudinal change that the Belfast Diet Study (1), UKPDS (4,18) and ADOPT

332     (6) could not identify, possibly due to differences in subject selection or to the use of post-MMTT

333     *vs* fasting insulin sensitivity indices. We also demonstrate that the associations between glycemic

334     deterioration and insulin sensitivity are independent from both the baseline value and the

335     progression rate of the *β*-cell function, and that insulin resistance progresses independently from *β*-

336     cell glucose sensitivity. Since in our analysis both HbA$_{1c}$ and insulin sensitivity trajectories were

337    adjusted for BMI changes and BMI did not increase on average, we can conclude that worsening of

338    insulin resistance in T2D and the associated glycemic deterioration are partly independent from

339    BMI changes. Whether the observed average increases in TG and AST (whose progression rates

340    were inversely correlated with OGIS progression rate) have a role in insulin sensitivity deterioration

341    (19), and whether this is mediated by ectopic fat accumulation (20), deserves further study.

342    UKPDS 25 and 26 (4,18), the Belfast Diet Study (1) and the ADOPT study (6) identified baseline

343    HOMA-%B as a predictor of glycemic deterioration (insulin requirement within 6 years for

344    UKPDS, time of failure to dietary therapy for the Belfast Diet Study, and monotherapy failure

345    before 4 years for ADOPT). Our study confirms the role of $\beta$-cell dysfunction as driver of glycemic

346    deterioration using a dynamic $\beta$-cell function assessment based on a glucose challenge, rather than

347    on fasting data only. We show that both baseline $\beta$-cell dysfunction (especially β-cell glucose

348    sensitivity) and its deterioration over time are independently associated with $HbA_{1c}$ worsening.

349    Moreover, we demonstrate that patients with limited or absent deterioration in $\beta$-cell function have

350    considerably lower odds of rapid glycemic deterioration.

351    Another novel finding is the strong and independent association between $HbA_{1c}$ progression and

352    insulin clearance during the MMTT, CLIm. To our knowledge, this is the first study examining

353    insulin clearance trajectories after T2D onset. We found that higher baseline CLIm and faster CLIm

354    increase over time independently associate with faster $HbA_{1c}$ progression. This is consistent with

355    the glucose homeostasis mechanisms, as higher CLIm reduces the average insulin levels. Notably,

356    we found a positive correlation between insulin sensitivity and insulin clearance, considering both

357    the baseline values of the two traits, in agreement with previous findings (21), and their progression

358    rates (Figure S2). However, on average, in spite of a decrease in insulin sensitivity, insulin

359    clearance did not decrease. These findings show that, while in pre-diabetic subjects insulin

360    clearance reduction may be a way to mitigate the effects of insulin resistance (22), in T2D patients

361    this compensation appears present but impaired and contributing to glycemic deterioration. The

362 reasons underlying these results remain elusive. The lack of decrease in insulin clearance may be

363 explained by the decrease of total MMTT insulin secretion and consequent desaturation of insulin

364 utilization (23) only in fast progressors, as in average progressors total insulin secretion slightly

365 increased (Figure 2). Whether hepatic or extrahepatic mechanisms underlie these findings cannot be

366 determined from this study and deserves further investigation.

367 Our results on TG and HDL effects were partially anticipated by a study of the Genetics of Diabetes

368 Audit and Research (GoDARTS) (24), where the outcome was the risk of progression to insulin

369 treatment. The study identified baseline TG and HDL (besides BMI, sex, and age, year and $HbA_{1c}$

370 at diagnosis) as independent determinants. A later study on the same data (25), investigating the

371 baseline determinants of $HbA_{1c}$ progression rate over about 9 years, confirmed an independent

372 effect of HDL (together with age, BMI and year at diagnosis) but not of TG. The FIELD study in

373 T2D patients on lifestyle measures only revealed that the HDL effect on initiation of oral

374 hypoglycemic agents survives the adjustment for HOMA-IR (26). Compared to previous studies

375 (24–26) our analysis includes the progression rates of plasma lipid components and baseline MRI

376 assessment of regional fat. We show that baseline HDL and BMI, and the progression rates of TG

377 and HDL are associated with $HbA_{1c}$ progression, even after accounting for the effects of the three

378 main determinants of glucose homeostasis, i.e. insulin sensitivity, β-cell function and insulin

379 clearance. In the subset of participants with MRI data, baseline visceral fat or liver fat was

380 independently correlated with $HbA_{1c}$ progression rate, a further novel observation. These findings

381 suggest that additional lipid-dependent factors contribute to $HbA_{1c}$ deterioration, possible

382 candidates being fat accumulation in the viscera (with excessive supply of fatty acids to the liver

383 (27)), liver fat and consequent hepatic insulin resistance (28), or glucose overproduction (29). The

384 role of visceral/liver fat supports interventions to reduce ectopic fat as a possible way for slowing

385 future glycemic progression.

386 Previous studies have reported an inverse correlation between baseline age and $HbA_{1c}$ progression

387 (1,4,6,24,25,30). In our analysis, baseline age does not have a clear independent role in the

388 multivariable model, most likely because the age range is relatively narrow relative to other studies,

389 or because the stronger predictors of $HbA_{1c}$ progression are correlated with age. The latter

390 explanation would suggest that the age univariate effect on glycemic deterioration is indirect. We

391 do not find a clear sex effect in glycemic deterioration, in agreement with most previous studies

392 (1,4,6,24,25).

393 In the multivariable model, baseline $HbA_{1c}$ was independently and inversely correlated with $HbA_{1c}$

394 progression rate, in contrast with previous findings (1,4,6,24,30). However, baseline $HbA_{1c}$ was not

395 significant in the logistic model. The most likely explanation of this finding is regression to the

396 mean: indeed, a random decrease in baseline $HbA_{1c}$ can produce a higher estimate of $HbA_{1c}$

397 progression rate, particularly when the follow-up period is not long, as in our study. Tight glycemic

398 control, an inclusion criterion, may have enhanced this effect.

399 This study does not find a relevant role of other variables often associated with glucose control. In

400 particular, we did not find an effect of smoking status (reported in GPRD (30)), T2D polygenic risk

401 score (in agreement with GoDARTS (24)), baseline values of diet, physical activity, pancreatic fat,

402 GLP-1, and glucagon. Several of these variables were not associated with $HbA_{1c}$ progression rate

403 even in simple correlation analysis (Figure S2). The lack of association for pancreatic fat is

404 particularly relevant, and contributes to the ongoing discussion on the role of pancreas fat in T2D

405 management (31).

406 In spite of the unique extensive phenotyping of our study and the consistent results, a significant

407 limitation is the relatively short follow-up period (3 years). The accuracy of the estimated $HbA_{1c}$

408 progression rate over this time frame may be limited, and in a longer time period the factors

409 contributing to progression may differ. In this study, we could not assess the changes over time of

410 relevant variables such as regional fat by MRI, diet and physical activity. MRI measurements were

411   available only for a subset of subjects. Insulin sensitivity was not derived from the gold standard

412   euglycemic clamp. As the cohort included only patients of white race, our findings are not

413   generalizable to other racial/ethnic groups. Causal relationships could not be inferred from our

414   regression analyses. The study of the mechanisms underlying the deterioration of the factors

415   affecting $HbA_{1c}$ progression, an important aspect to envisage optimal treatment strategies, also

416   requires further investigation.

417   In summary, based on the extensively phenotyped cohort of white European diabetic patients of the

418   DIRECT study, we identified decreasing insulin sensitivity, deteriorating β-cell function, increasing

419   insulin clearance, high liver or visceral fat, and worsening of the lipid profile as the most important

420   factors independently associated with $HbA_{1c}$ deterioration in the early phase of the disease. We also

421   showed that patients with a relatively stable value over time of at least one of insulin sensitivity, β-

422   cell glucose sensitivity, or insulin clearance have considerably reduced odds of fast $HbA_{1c}$ increase.

423   This study contributes to the understanding of the factors underlying diabetes progression,

424   elucidating the processes that might be targeted for personalized treatments.

449    Eli Lilly, Novo Nordisk and Zoe Global Ltd; he also reports stock options in Zoe Global Ltd. All

450    other co-authors have nothing to disclose.

451    **Author Contributions.**

452    R.B. and A.M. designed the analysis, analyzed the data, and wrote the manuscript. R.B., C.J.,

453    A.G.J., M.W., E.R.P. and A.M. interpreted the results. E.R.P. and A.M. supervised the analysis.

454    C.J., A.G.J., A.K., M.W. and E.R.P. reviewed the manuscript. All authors were involved in the

455    DIRECT study at different levels, and were essential for the production, release and management of

456    the data analyzed here. R.B. is the guarantor of this work and, as such, takes full responsibility for

457    the work as a whole, including the study design, access to data, and the decision to submit and

458    publish the manuscript.

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546 **Table 1.** Proportion of fast HbA1c progressors with different combinations of stable/deteriorating conditions for GS, OGIS and CLIm progression
547 rates.

| Condition[*] | | | Average progressors (N) | Fast progressors (N) | Fast progressors (%) [95% CI] | Odds ratio [95% CI] | p-value[†] |
|---|---|---|---|---|---|---|---|
| **GS** | **OGIS** | **CLIm** | | | | | |
| Deteriorating | Deteriorating | Stable | 47 | 5 | 9.6 [4.2,20.6] | 0.09 [0.02,0.32] | 2E-4 |
| Deteriorating | Stable | Deteriorating | 56 | 6 | 9.7 [4.5,19.5] | 0.09 [0.02,0.30] | 8E-5 |
| Stable | Deteriorating | Deteriorating | 34 | 3 | 8.1 [2.8,21.3] | 0.07 [0.02,0.32] | 4E-4 |
| Deteriorating | Deteriorating | Deteriorating | 8 | 10 | 55.6 [33.7,75.4] | - | - |

548 [*] The progression rate thresholds dividing stable and deteriorating traits for OGIS, GS and CLIm are -16.68 ml min$^{-1}$ m$^{-2}$ year$^{-1}$, -4.07 pmol min$^{-1}$ m$^{-2}$ mmol$^{-1}$ l
549 year$^{-1}$ and 0.0184 l min$^{-1}$ m$^{-2}$ year$^{-1}$, respectively.
550 [†] Two-sided Chi-square test ($\alpha$=0.05), with Yates continuity correction, on the proportion of fast progressors in the row compared to the same proportion in the
551 last row.
552 GS: $\beta$-cell glucose sensitivity; OGIS: oral insulin sensitivity; CLIm: mixed meal test insulin clearance.

**Figure legends**

554  Figure 1. Variables independently associated with $HbA_{1c}$ progression rate from multivariable linear

555  analysis. Panel A: all subjects are included in the analysis (625 with all variables), and MRI

556  measurements are not considered; panel B: only subjects with MRI are included in the analysis (374

557  with all variables), and MRI measurements are taken into consideration. For each variable, the

558  figure shows the standardized coefficients ± 95% CI of the effect. Age and HDL were log-

559  transformed. OGIS: oral insulin sensitivity; CLIm: mixed meal test insulin clearance; GS: $\beta$-cell

560  glucose sensitivity; TG: fasting triacylglycerol; HDL: fasting HDL-cholesterol; RS: $\beta$-cell rate

561  sensitivity; progr: progression rate; bas: baseline value; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$.

562  Figure 2. Temporal trajectories or baseline values (bar graphs) of $HbA_{1c}$ and other key traits in fast

563  (red lines) and average (blue lines) progressors. Data are mean ± standard error. Simple

564  comparisons between fast and average progressors (Wilcoxon rank sum test) are shown for baseline

565  values (asterisks at month 0) and progression rates (asterisks at month 18). These comparisons may

566  differ from the results of the multivariable analyses (Figures 2 and 4). Sex is not included in the

567  figure: males were 42% and 36% in average and fast progressors, respectively (non-significant,

568  Chi-squared test). $HbA_{1c}$ values at 27 months are not displayed as they were collected in a subgroup

569  of individuals. In average progressors, $HbA_{1c}$ increases from 46.4±0.2 mmol/mol to 46.7±0.3

570  mmol/mol; in fast progressors, from 48.9±1.21 mmol/mol to 75.7±2.5 mmol/mol. OGIS: insulin

571  sensitivity; CLIm: mixed meal test insulin clearance; GS: $\beta$-cell glucose sensitivity; RS: $\beta$-cell rate

572  sensitivity; TG: fasting triacylglycerol; HDL: fasting HDL-cholesterol; ISRtot: total mixed meal

573  test insulin secretion; bas: baseline value; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$.

574  Figure 3. Odds ratios ± 95% CI from the multivariable logistic analysis of fast *vs* average $HbA_{1c}$

575  progressors. The independent variables are those identified by multivariable linear analysis of

576  $HbA_{1c}$ progression, excluding MRI variables (*N*=625, with 32 fast progressors and 593 average

577  progressors). Age and HDL were log-transformed. Values for sensitivity, specificity and accuracy

578    were derived via maximization of balanced accuracy. OGIS: insulin sensitivity; CLIm: mixed meal

579    test insulin clearance; GS: $\beta$-cell glucose sensitivity; TG: fasting triacylglycerol; HDL: fasting

580    HDL-cholesterol; RS: $\beta$-cell rate sensitivity; progr: progression rate; bas: baseline value; AUROC:

581    area under the receiver operating characteristics; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$.