



**University of Dundee**

## **Processes Underlying Glycemic Deterioration in Type 2 Diabetes**

IMI DIRECT Consortium; Bizzotto, Roberto; Jennison, Christopher; Jones, Angus G.; Kurbasic, Azra; Tura, Andrea

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2 Processes underlying glycemetic deterioration in type 2 diabetes: An IMI DIRECT study

3

4 **Running title**

5 Glycemic deterioration in type 2 diabetes

6

7 **Author names**

8 Roberto Bizzotto<sup>1,\*</sup>, Christopher Jennison<sup>2</sup>, Angus G Jones<sup>3</sup>, Azra Kurbasic<sup>4</sup>, Gwen Kennedy<sup>5</sup>,  
9 Jimmy D Bell<sup>6</sup>, Elizabeth L Thomas<sup>6</sup>, Gary Frost<sup>7</sup>, Rebeca Eriksen<sup>7</sup>, Robert W Koivula<sup>4,8</sup>, Soren  
10 Brage<sup>9</sup>, Jane Kaye<sup>10,11</sup>, Andrew T Hattersley<sup>3</sup>, Alison Heggie<sup>12</sup>, Donna McEvoy<sup>13</sup>, Leen M 't  
11 Hart<sup>14,15,16</sup>, Joline W Beulens<sup>14</sup>, Petra Elders<sup>17</sup>, Petra B Musholt<sup>18</sup>, Martin Ridderstråle<sup>19</sup>, Tue H  
12 Hansen<sup>20</sup>, Kristine H Allin<sup>20</sup>, Torben Hansen<sup>20</sup>, Henrik Vestergaard<sup>20,21</sup>, Agnete T Lundgaard<sup>22</sup>,  
13 Henrik S Thomsen<sup>23</sup>, Federico De Masi<sup>24</sup>, Konstantinos D Tsirigos<sup>22,25</sup>, Søren Brunak<sup>22,25</sup>, Ana  
14 Viñuela<sup>26</sup>, Anubha Mahajan<sup>27,a</sup>, Timothy J McDonald<sup>3,28</sup>, Tarja Kokkola<sup>29</sup>, Ian M Forgie<sup>30</sup>,  
15 Giuseppe N Giordano<sup>4</sup>, Imre Pavo<sup>31</sup>, Hartmut Ruetten<sup>18</sup>, Emmanouil Dermitzakis<sup>26</sup>, Mark I  
16 McCarthy<sup>8,27,32,a</sup>, Oluf Pedersen<sup>20</sup>, Jochen M Schwenk<sup>33</sup>, Jerzy Adamski<sup>34,35,36</sup>, Paul W Franks<sup>4</sup>,  
17 Mark Walker<sup>37</sup>, Ewan R Pearson<sup>30</sup>, Andrea Mari<sup>1</sup> for the IMI DIRECT consortium<sup>38</sup>.

18

19 **Affiliations**

20 <sup>1</sup> CNR Institute of Neuroscience, Padova, Italy;

21 <sup>2</sup> Department of Mathematical Sciences, University of Bath, Bath, United Kingdom;

22 <sup>3</sup> Institute of Clinical and Biological Sciences, University of Exeter Medical School, Exeter, United  
23 Kingdom;

24 <sup>4</sup> Genetic and Molecular Epidemiology Unit, Lund University Diabetes Centre, Department of  
25 Clinical Sciences, CRC, Lund University, SUS, Malmö, Sweden;

26 <sup>5</sup> Immunoassay Biomarker Core Laboratory, School of Medicine, Ninewells Hospital, Dundee,  
27 United Kingdom;

28 <sup>6</sup> Research Centre for Optimal Health, Department of Life Sciences, University of Westminster,  
29 London, United Kingdom;

30 <sup>7</sup> Section for Nutrition Research, Faculty of Medicine, Hammersmith Campus, Imperial College  
31 London, London, United Kingdom;

32 <sup>8</sup> Oxford Centre for Diabetes, Endocrinology and Metabolism, Radcliffe Department of Medicine,  
33 University of Oxford, Oxford, United Kingdom;

34 <sup>9</sup> MRC Epidemiology Unit, University of Cambridge, United Kingdom;

35 <sup>10</sup> Centre for Health, Law and Emerging Technologies (HeLEX), Faculty of Law, University of  
36 Oxford, Oxford, United Kingdom;

37 <sup>11</sup> Centre for Health, Law and Emerging Technologies (HeLEX), Melbourne Law School,  
38 University of Melbourne, Carlton, Victoria, Australia;

39 <sup>12</sup> Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom;

40 <sup>13</sup> Diabetes Research Network, Royal Victoria Infirmary, Newcastle upon Tyne, United Kingdom;

41 <sup>14</sup> Department of Epidemiology & Biostatistics, Amsterdam UMC - location VUmc, Amsterdam  
42 Public Health Research Institute, Amsterdam, The Netherlands;

43 <sup>15</sup> Department of Cell and Chemical Biology, Leiden University Medical Center, Leiden, the  
44 Netherlands;  
45 <sup>16</sup> Department of Biomedical Data Sciences, Molecular Epidemiology section, Leiden University  
46 Medical Center, Leiden, the Netherlands;  
47 <sup>17</sup> Department of General Practice, Amsterdam UMC - location VUmc, Amsterdam Public Health  
48 Research Institute, Amsterdam, The Netherlands;  
49 <sup>18</sup> R&D Global Development, Translational Medicine & Clinical Pharmacology (TMCP), Sanofi-  
50 Aventis Deutschland GmbH, Frankfurt, Germany;  
51 <sup>19</sup> Clinical Pharmacology and Translational Medicine, Novo Nordisk A/S, Søborg, Denmark;  
52 <sup>20</sup> Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health and Medical  
53 Sciences, University of Copenhagen, Denmark;  
54 <sup>21</sup> Bornholms Hospital, Rønne, Denmark;  
55 <sup>22</sup> Novo Nordisk Foundation Center for Protein Research, University of Copenhagen, Copenhagen,  
56 Denmark;  
57 <sup>23</sup> Faculty of Medical and Health Sciences, University of Copenhagen, Copenhagen;  
58 <sup>24</sup> Department of Health Technology, Technical University of Denmark, Kongens Lyngby,  
59 Denmark;  
60 <sup>25</sup> Center for Biological Sequence Analysis, Department of Systems Biology, Technical University  
61 of Denmark, Kongens Lyngby, Denmark;  
62 <sup>26</sup> Department of Genetic Medicine and Development, University of Geneva Medical School,  
63 Geneva, Switzerland;  
64 <sup>27</sup> Wellcome Centre for Human Genetics, University of Oxford, Oxford, United Kingdom;  
65 <sup>28</sup> Blood Sciences, Royal Devon and Exeter NHS Foundation Trust, Exeter, United Kingdom;  
66 <sup>29</sup> Department of Medicine, University of Eastern Finland, Kuopio, Finland;  
67 <sup>30</sup> Population Health & Genomics, School of Medicine, University of Dundee, Dundee, United  
68 Kingdom;  
69 <sup>31</sup> Eli Lilly Regional Operations GmbH, Vienna, Austria;  
70 <sup>32</sup> Oxford NIHR Biomedical Research Centre, Oxford University Hospitals NHS Foundation Trust,  
71 John Radcliffe Hospital, Oxford, United Kingdom;  
72 <sup>33</sup> Affinity Proteomics, Science for Life Laboratory, School of Engineering Sciences in Chemistry,  
73 Biotechnology and Health, KTH - Royal Institute of Technology, Solna, Sweden;  
74 <sup>34</sup> Research Unit of Molecular Endocrinology and Metabolism, Helmholtz Zentrum München,  
75 Neuherberg, Germany;  
76 <sup>35</sup> Lehrstuhl für Experimentelle Genetik, Technische Universität München, Freising-  
77 Weihenstephan, Germany;  
78 <sup>36</sup> Department of Biochemistry, Yong Loo Lin School of Medicine, National University of  
79 Singapore, Singapore, Singapore;  
80 <sup>37</sup> Translational and Clinical Research Institute, Faculty of Medical Sciences, Newcastle University,  
81 Newcastle, United Kingdom;  
82 <sup>38</sup> Names and affiliations listed in the Supplemental Material.  
83 <sup>a</sup> Current address: Genentech, 1 DNA Way, South San Francisco, CA 94080  
84

85 **\*Corresponding author**

86 Roberto Bizzotto

87 Corso Stati Uniti 4

88 35127 Padova

89 Italy

90 Telephone: 0039 049 8295796

91 Fax: 0039 049 8295763

92 Email address: [roberto.bizzotto@cnr.it](mailto:roberto.bizzotto@cnr.it)

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95

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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),  $\beta$ -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<sub>1c</sub>  
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<sub>1c</sub> 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  $\beta$ -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,  $\beta$ -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 glyceic progression can be  
122 supported by understanding the processes underlying deterioration of glucose control.

123 Few studies have assessed HbA<sub>1c</sub> trajectories and the possible determinants of glyceic  
124 deterioration. An established finding is that  $\beta$ -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  $\beta$ -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<sub>1c</sub> 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 glyceic  
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<sub>1c</sub>,  $\beta$ -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<sup>2</sup>, estimated  
149 glomerular filtration rate >50 ml/min, and HbA<sub>1c</sub> 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<sub>1c</sub> 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<sub>1c</sub>, blood lipids and liver enzymes were collected at all visits. A 27-month  
157 HbA<sub>1c</sub> 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)),  $\beta$ -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<sub>1c</sub> 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<sub>1c</sub>  
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<sub>1c</sub> 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 HbA<sub>1c</sub> 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  $\pm$  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<sub>1c</sub>  
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 were 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<sub>1c</sub> 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<sub>1c</sub> 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  $62 \pm 8$  years, were moderately obese ( $30.4 \pm 4.9$  kg/m<sup>2</sup> BMI),  
212 and had HbA<sub>1c</sub> of  $6.41 \pm 0.53$  % ( $46.5 \pm 5.8$  mmol/mol) and fasting glucose of  $7.1 \pm 1.4$  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<sub>1c</sub> and other traits*

215 The individual HbA<sub>1c</sub> 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<sup>-1</sup> year<sup>-1</sup>), respectively). However, the distribution showed a heavy right tail with values  
219 up to 0.897 %/year (9.8 mmol mol<sup>-1</sup> year<sup>-1</sup>). 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<sub>1c</sub> progression rate and laboratory,  
229 clinical, and functional parameters (Supplemental Figure S2). In particular, HbA<sub>1c</sub> 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<sub>1c</sub> progression rate being associated with both of them.

238 *Variables associated with HbA<sub>1c</sub> progression rate: multivariable linear analysis*

239 In multivariable linear analysis of HbA<sub>1c</sub> 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<sub>1c</sub> progression was independently associated with lower baseline values and  
242 faster deterioration of insulin sensitivity (as OGIS) and  $\beta$ -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<sub>1c</sub> 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<sub>1c</sub> ( $p<0.001$ ). 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<sub>1c</sub> progression rate; moreover, female sex and younger age independently predicted faster  
253 HbA<sub>1c</sub> 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<sub>1c</sub> 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<sub>1c</sub> progression rate: multivariable logistic analysis*

263 The threshold selected to separate the heavy right tail of the distribution of HbA<sub>1c</sub> progression rates  
264 was 0.255 %/year (2.79 mmol mol<sup>-1</sup> year<sup>-1</sup>). This threshold split the subjects into average  
265 progressors ( $N=699$ ), with a progression rate of  $0.044\pm 0.076$  %/year ( $0.48\pm 0.83$  mmol mol<sup>-1</sup> year<sup>-1</sup>),  
266 and fast progressors ( $N=33$ ), with a ~10-fold mean progression rate ( $0.460\pm 0.185$  %/year,  
267  $5.03\pm 2.02$  mmol mol<sup>-1</sup> year<sup>-1</sup>) (Figure 2).

268 We found that the trajectories of most variables independently affecting HbA<sub>1c</sub> 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<sub>1c</sub> 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<sub>1c</sub> 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<sub>1c</sub> progressors*

287 Because HbA<sub>1c</sub> 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 glycemc  
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 glycemc deterioration in T2D patients in the initial phase of the  
304 disease. We found that HbA<sub>1c</sub> deterioration was independently associated with 1) a decrease in  
305 insulin sensitivity; 2) a decrease in  $\beta$ -cell function (primarily  $\beta$ -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<sub>1c</sub> 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<sub>1c</sub>  
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<sub>1c</sub> 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<sub>1c</sub> progression of several variables, in particular the  
317 progression rates of insulin sensitivity,  $\beta$ -cell function and insulin clearance, and the existence of  
318 fast HbA<sub>1c</sub> 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  $\beta$ -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  $\beta$ -cell function, and that insulin resistance progresses independently from  $\beta$ -  
336 cell glucose sensitivity. Since in our analysis both HbA<sub>1c</sub> 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 glyceemic 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 glyceemic 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 glyceemic  
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  $\beta$ -cell glucose  
348 sensitivity) and its deterioration over time are independently associated with HbA<sub>1c</sub> worsening.  
349 Moreover, we demonstrate that patients with limited or absent deterioration in  $\beta$ -cell function have  
350 considerably lower odds of rapid glyceemic deterioration.

351 Another novel finding is the strong and independent association between HbA<sub>1c</sub> 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<sub>1c</sub> 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 glyceemic 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<sub>1c</sub>  
370 at diagnosis) as independent determinants. A later study on the same data (25), investigating the  
371 baseline determinants of HbA<sub>1c</sub> 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<sub>1c</sub> progression, even after accounting for the effects of the three  
378 main determinants of glucose homeostasis, i.e. insulin sensitivity,  $\beta$ -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<sub>1c</sub> progression rate, a further novel observation. These findings  
381 suggest that additional lipid-dependent factors contribute to HbA<sub>1c</sub> 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 glycaemic progression.

386 Previous studies have reported an inverse correlation between baseline age and HbA<sub>1c</sub> 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<sub>1c</sub> 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<sub>1c</sub> was independently and inversely correlated with HbA<sub>1c</sub>  
394 progression rate, in contrast with previous findings (1,4,6,24,30). However, baseline HbA<sub>1c</sub> 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<sub>1c</sub> can produce a higher estimate of HbA<sub>1c</sub>  
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<sub>1c</sub> 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<sub>1c</sub>  
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<sub>1c</sub> 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  $\beta$ -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<sub>1c</sub> 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,  $\beta$ -  
422 cell glucose sensitivity, or insulin clearance have considerably reduced odds of fast HbA<sub>1c</sub> 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.

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435 **Duality of Interest.**

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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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- 545



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  $-16.68 \text{ ml min}^{-1} \text{ m}^{-2} \text{ year}^{-1}$ ,  $-4.07 \text{ pmol min}^{-1} \text{ m}^{-2} \text{ mmol}^{-1} \text{ l}$   
 549  $\text{year}^{-1}$  and  $0.0184 \text{ l min}^{-1} \text{ m}^{-2} \text{ year}^{-1}$ , 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.

553 **Figure legends**

554 Figure 1. Variables independently associated with HbA<sub>1c</sub> 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  $\pm$  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<sub>1c</sub> and other key traits in fast  
563 (red lines) and average (blue lines) progressors. Data are mean  $\pm$  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<sub>1c</sub> values at 27 months are not displayed as they were collected in a subgroup  
569 of individuals. In average progressors, HbA<sub>1c</sub> increases from  $46.4 \pm 0.2$  mmol/mol to  $46.7 \pm 0.3$   
570 mmol/mol; in fast progressors, from  $48.9 \pm 1.21$  mmol/mol to  $75.7 \pm 2.5$  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  $\pm$  95% CI from the multivariable logistic analysis of fast vs average HbA<sub>1c</sub>  
575 progressors. The independent variables are those identified by multivariable linear analysis of  
576 HbA<sub>1c</sub> 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; \*:  $p < 0.05$ ; \*\*:  $p < 0.01$ ; \*\*\*:  $p < 0.001$ .