Machine Learning in Multi-Omics Data to Assess Longitudinal Predictors of Glycaemic Health

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Abstract

Type 2 diabetes (T2D) is a global health burden that will benefit from personalised risk prediction and targeted prevention programmes. Omics data have enabled more detailed risk prediction; however, most studies have focussed on directly on the ability of DNA variants predicting T2D onset with less attention given to epigenetic regulation and glycaemic trait variability. By applying machine learning to the longitudinal Northern Finland Birth Cohort 1966 (NFBC 1966) at 31 (T1) and 46 (T2) years old, we predicted fasting glucose (FG) and insulin (FI), glycated haemoglobin (HbA1c) and 2-hour glucose and insulin from oral glucose tolerance test (2hGlu, 2hIns) at T2 in 513 individuals from 1,001 variables at T1 and T2, including anthropometric, metabolic, metabolomic and epigenetic variables. We further tested whether the information obtained by the machine learning models in NFBC could be used to predict glycaemic traits in the independent French study with 48 matching predictors (DESIR, N=769, age range 30-65 years at recruitment, interval between data collections: 9 years). In this study, FG and FI were best predicted, with average R^2 values of 0.38 and 0.53. Sex, branched-chain and aromatic amino acids, HDL-cholesterol, glycerol, ketone bodies, blood pressure at T2 and measurements of adiposity at T1, as well as multiple methylation marks at both time points were amongst the top predictors. In the validation analysis, we reached R^2 values of 0.41/0.55 for FG/FI when trained and tested in NFBC1966 and 0.17/0.30 when trained in NFBC1966 and tested in DESIR. We identified clinically relevant sets of predictors from a large multi-omics dataset and highlighted the potential of methylation markers and longitudinal changes in prediction.

Key Words

Glycaemic traits, longitudinal, machine learning, metabolomics, methylation, prediction, type 2 diabetes

Background

Diabetes accounts for the yearly deaths of about four million people between 20 and 79 years old (2017) world-wide and the prevalence of diabetes is expected to increase from 8.8% to 9.9% by 2045.[1] Moreover, glucose tolerance impairment is progressing in young individuals, leading to high risk of developing type 2 diabetes (T2D) later in life.[1]

To date, T2D risk prediction in the clinical practice has focussed on the classical risk factors of sex, age, obesity, family history, hypertension, cholesterol levels and lifestyle factors [2–5]. Recent advances in omics technologies have allowed exploring the risk factors of T2D in more detail, opening possibilities for more precise biomarkers and thus, better identification of people at risk for T2D in the future. A large number of metabolites, including amino acids, especially branched-chain amino acids (BRACA) and aromatic amino acids, fatty acids, glycerophospholipids, ketone bodies and mannose have been associated with T2D incidence[6–8]. However, whether metabolites can be effective and reliable T2D predictors, remains unclear.

During the past decade, genome-wide association studies (GWAS) have enlightened the genetic risk of developing T2D. Currently, 403 independent DNA variants are established for T2D risk by GWAS meta-analyses[9,10] as well as dozens of loci have been associated with quantitative glycaemic traits in individuals without T2D, including fasting glucose (FG)[11], fasting insulin (FI)[11], FG adjusted for body-mass-index (BMI)[11], FI adjusted for BMI[11], 2 hour post-prandial or post oral glucose-tolerance test glucose (2hGluc) [12] and glycaeted haemoglobin (HbA1c)[13]. Besides genetics, environment and lifestyle are likely to have a large contribution to T2D risk[14]. Environmental factors can affect gene expression by an addition of a methyl group on a CpG-dinucleotide site of DNA. This is called DNA methylation and is the most widely studied type of epigenetic modification[15,16]. Studies in peripheral blood have found a mean absolute difference of 0.5-1.1% in methylation levels between individuals with and without T2D[17]. Epigenome-wide association studies have reported associations at 65 methylation

96 markers for T2D[17,18] and provided support for overlap in epigenetic effects between T2D and glycaemic
97 traits[18,19]. The epigenetic effect on variability of glycaemic traits is magnified in the presence of
98 obesity[19]. The investigation of the link between BMI and methylation levels demonstrates that
99 methylation at the majority of CpG sites in blood is consequential to higher BMI[20]. Interestingly, a
100 weighted methylation risk score calculated from 187 markers was shown to have an even stronger
101 effect on incident T2D[20] than the traditional risk factors of overweight, central obesity, phenylalanine,
102 tyrosine, isoleucine, FG, FI and C-reactive protein. The methylation risk score remained associated with
103 incident T2D even after adjustment for age, sex, BMI, FI, FG and central obesity[20].
104
105 In the era of multi-omics data and millions of measurable variables, it is challenging to identify the best
106 biomarkers for clinical use. Machine learning approaches are ideal for such high-dimensional data due
107 to their ability to learn from the data and identify patterns without knowledge of the joint distribution
108 of the variables[21]. Recently, the studies of T2D risk have leveraged association analyses as well as
109 machine learning algorithms for the prediction of binary T2D phenotypes. Thus far, machine learning
110 used for T2D classification include logistic regression with and without Lasso regularization[17,20,22–26],
111 Regularized least-squares (RLS)[27], Cox regression[23], naïve Bayes[25] and J48-decision tree[25]. In predictive
112 studies using machine learning models, classical risk factors, genetic risk scores (GRS)[22], methylation
113 risk scores (MRS)[17,20] and metabolomic data[24–27] have been used as predictors of T2D incidence after a
114 follow-up window of two to fourteen years. A few studies have suggested that metabolites improve
115 prediction performance[23,25–27], while others have reported negligible to no improvement in
116 prediction[24]. GRS have been shown to bring no incremental value over classical non-invasive factors
117 and metabolic markers[22]. MRS combining CpG loci have been found to be associated with future T2D
118 incidence[17,20]. All the previous studies have focused on the binary disease status as the outcome which
119 may greatly reduce the power of the analyses. To our knowledge, there are no machine learning
120 studies on predictors of continuous glycaemic traits relevant for T2D pathophysiology. In addition,
121 most of the studies on T2D have used a single pre-selected machine learning approach and have not

122 compared their performance in terms of predictive capacities. With the present study, we aimed to
123 address these shortcomings, as well as to shed light on the contribution of longitudinal predictors,
124 especially metabolomic and methylation markers by using data from two well-characterised
125 population-based studies and by applying and comparing six different machine learning approaches.
126

127 ## Results

128 We focused on epigenetic and metabolic markers (**Supplementary Table 1**, **Supplementary Table 2**)
129 from the Northern Finland Birth Cohort 1966 (NFBC1966) measured at 31 (T1) and 46 (T2) years for
130 prediction of HbA1c, FG, 2hGluc, FI and two-hours post-oral glucose tolerance test insulin (2hIns) at T2
131 in individuals free from T2D diagnosis or medication at T1. We implemented and compared the results
132 obtained from six machine learning approaches: Boosted trees (BT), Random forest (RF) and support
133 vector regression (SVR) with Linear Kernel with L2 regularization and with L1 and L1/L2 loss functions
134 (SVR-L2Linear-L1, SVR-L2Linear-L1L2, respectively), with Polynomial Kernel (SVR-Polynomial) and with
135 Radial Basis function Kernel (SVR-RBF). The algorithms were chosen for their ability to handle a large
136 number of predictors, to account for multi-collinearity, non-linear relationships, the absence of the
137 assumption regarding data distribution, and for their computational times. We also tested different
138 input data combinations (**Figure 1**). We further validated our approach in an independent French
139 cohort (Data from an Epidemiological Study on the Insulin Resistance syndrome, DESIR) that shared
140 48 variables with the NFBC1966 cohort (**Supplementary Table 3**).
141

142 **Best predicted outcome variables**

143 We compared the performance of the six models for each of the outcomes (HbA1c, FG, 2hGluc, FI and
144 2hIns) with varying input data combinations, including omics data in their raw or scored forms
145 (**Methods, Supplementary Table 4**). With the usage of metabolic data as the minimal input, we
146 observed that performance was the best for FI prediction (**Figure 2A**). In the context of the model with

147 metabolic raw data (Mb-R), models with different outcomes were ranked as follows: FI > FG > 2hIns >
148 2hGlc > HbA1c ($P_{TukeyHSD}$<5.0x10^{-5} for all other comparisons except for 2hIns>2hGluc for which
149 $P_{TukeyHSD}$=0.19, **Supplementary Table 5**). The average coefficients of determination R^2 over all machine
150 learning algorithms were 0.53, 0.38, 0.29, 0.25 and 0.14 (Mb-R) for FI, FG, 2hIns, 2hGlc, HBA1c
151 respectively (**Supplementary Table 6**).
152
153 **Best predictor data combinations**
154 Regarding the predictors, we found that models that included at least some metabolic data, either in
155 their raw format (Mb-R) or transformed into scores (Mb-S) (**Methods**) had the best performance
156 reaching a maximum R^2=0.56 (**Figure 2A, Supplementary Table 7**). In contrast, models with
157 methylation data only as predictors (Mh-R and Mh-S), reached R^2 values of up to 0.20. Thus, metabolic
158 models performed significantly better than pure epigenomic models ($P_{TukeyHSD}$<1.7×10^{-9}) (**Table 1**,
159 Comparison 1-4; all comparisons are provided in **Supplementary Table 8**). When metabolic and
160 methylation data were combined, Mb-R performed better for FI and FG than Mb-R + Mh-R ($P_{TukeyHSD}$
161 <0.04). Adding Mh-S to the model did not alter the model performance for any of the outcomes
162 ($P_{TukeyHSD}$>0.99) (**Table 1**, Comparison 5-6). These results suggest that addition of methylation
163 information does not increase the predictive ability of the tested models. When exploring the effect
164 of transforming the original variables into scores (**Table 1**, comparison 7-8), we observed no significant
165 differences for any of the outcomes when comparing Mb-R vs. Mb-S ($P_{TukeyHSD}$>0.29). This was true also
166 for Mh-R vs. Mh-S, except for the prediction of FI, where Mh-R performed better than Mh-S
167 ($P_{TukeyHSD}$=1.6×10^{-4}). Therefore, based on these results we are unable to generalise better performance
168 of the scored data as compared to raw data. Finally, we found that Mb-S + Mh-S model performed
169 significantly better than the model with Mb-S + Mh-R ($P_{TukeyHSD}$<0.02) for all outcomes except for
170 2hGluc. This observation reflects the decrease in performance of the models upon inclusion of a large
171 number of weak predictors.
172

173 **Adjustment for Measures of Adiposity**

174 To understand the influence of the measures of adiposity in the models, we adjusted all the outcomes
175 for T1-BMI, T2-BMI, T1-WHR and T2-WHR and performed the machine learning models on these
176 adjusted outcomes. All adjusted models exhibited an R^2<0.13 (**Figure 2B, Supplementary Figure 1** with
177 a zoomed-in scale for R^2, **Supplementary Table 9**), including models predicting FI and FG. Therefore,
178 the measures of adiposity at T1 and T2 are the main drivers of prediction for FI and FG.

179

180 **Variable Importance**

181 We investigated the contribution of metabolic and epigenomic variables to the prediction of glycaemic
182 traits. We discuss predictors importance only in the context of FG and FI outcomes, for which
183 prediction algorithms reached the best R^2 (**Figure 2A**). FG prediction was mostly explained by metabolic
184 variables: valine, leucine, isoleucine, tyrosine, BMI and WHR, HDLs and VLDL, glycerol, alanine, SBP and
185 DBP at T2, WHR and FG at T1 and sex (**Figure 3** bottom right). FI prediction was explained by BMI, WHR,
186 HDL, VLDL, BRACA, phenylalanine, leucine, glycerol, lactate, tyrosine, valine at T2, and FI at T1 (**Figure**
187 **3** top left). Once we adjusted the outcomes for the measures of adiposity, the top predictor for FI was
188 measurement of FI at T1, followed by alanine and other variables at T2 already observed as important
189 before adjustment for measurements of adiposity (**Supplementary Figure 2**). The top variables driving
190 the prediction of FG were, similarly to FI, valine at T2 and FG at T1 (**Supplementary Figure 2**). The
191 metabolic models with scored variables were driven by variables that mirrored the top raw predictors.
192 Overall, the model with scored variables for FI supported the importance of the former variables, as
193 well as ketone bodies (acetoacetate and 3-hydroxybutyrate) at T2 (**Figure 3**, top right and bottom left).
194 Importantly, even though we previously observed that adding methylation data does not improve
195 model prediction, a number of methylation probes were among the top predictors for both FG and FI
196 when combined with scored metabolic variables (**Figure 3**).

197

198 **Association Analysis for Effect Sizes, Direction and Variance Explained**

199 Linear regression analyses between ln(FI)/FG and each of the top 25 predictors identified by machine
200 learning indicated that increases in HDL cholesterol were predictive of decreased values of FI and FG
201 (**Table 2**). The same was true for free cholesterol to total lipids ratio in IDL and cholesterol esters to
202 total lipids ratio in medium VLDL. Similarly, female gender and higher sum score of ketone bodies, i.e.
203 acetoacetate and 3-hydroxybutyrate, predicted lower FI and FG levels, as did higher methylation levels
204 of the probes T2_cg00574958, T2_cg17058475, and T2_cg08309687. In the models with scored
205 variables, the adjusted R^2 for ln(FI) when including all 25 top predictors was 0.58. After exclusion of
206 methylation markers, it decreased to 0.54 and finally, after excluding all other variables than measures
207 of adiposity, the variance explained was 0.40. The same figures for FG were 0.45, 0.42 and 0.31,
208 respectively.
209
210 **Prediction from Variables at T1**
211 To test whether variables already 15 years beforehand, i.e. at T1 only, can provide information about
212 glycaemic traits at T2, we restricted the prediction variables to those measured at T1 only. For
213 example, under the well-performing Mb-R in the full model with predictors both from T1 and T2 and
214 unadjusted for measures of adiposity, FI, FG, 2hIns, 2hGlc, HBA1c were predicted with R^2 values of
215 0.53, 0.38, 0.29, 0.25 and 0.14, respectively. The restriction to T1 variables caused a drop in R^2 to 0.25,
216 0.22, 0.15, 0.06, 0.06, respectively, when averaging over all machine learning methods. This suggests
217 that prediction from T1 variables only is not achievable in our dataset.
218
219 **Performance of the machine learning algorithms**
220 When at least metabolic data in either form (Mb-R or -S + any other data) were included as input, we
221 found no statistically significant differences between the performances of RF and BT for all phenotypes
222 ($P_{TukeyHSD}$>0.91, **Supplementary Table 10**). In addition, no significant difference was found between
223 SVR-L2Linear-L1L2 and RF or BT models ($P_{TukeyHSD}$>0.29, **Supplementary Table 10**). Among SVR models,
224 we found that SVR-L2Linear-L1L2 either performed equally or outperformed the other SVRs,

225 depending on the input dataset. In particular, for datasets with a large number of predictors SVR-
226 L2Linear-L1L2 was the best performing SVR ($P_{TukeyHSD}$<0.05, **Supplementary Table 10**). SVR-L2Linear-L1
227 in turn showed lower performance ($P_{TukeyHSD}$<0.05, **Supplementary Table 10**) at several occasions when
228 compared to that of the other algorithms. Even though both SVR-L2Linear-L1 and SVR-L2Linear-L1L2
229 both use the L2 regularization, the first uses only the L1 loss function whereas the latter optimizes over
230 both L1 and L2 loss functions (**Supplementary Material**). We observed that in 78.5% of the time the
231 L2 loss function was chosen over L1 in the SVR-L2Linear-L1L2 analyses (data not shown). We
232 investigated whether the better performance of the SVR-L2Linear-L1L2 algorithm over the SVR-
233 L2Linear-L1 was due to the evaluation criterion used, namely R^2 which computes a scaled measure
234 based on the quadratic loss function. For this purpose, we assessed the model performance in terms
235 of Mean Absolute Error (MAE), i.e. based on L1 loss. By definition we aim to maximize R^2 while we aim
236 to minimize MAE. Evaluations based on MAE did not show improved performance of the predictions
237 based on SVR-L2Linear-L1 (**Supplementary Figure 3**, **Supplementary Table 11**).
238
239 **Validation of the Machine Learning models in the French DESIR cohort**
240 For the replication analysis, we first trained and tested the data in the NFBC1966 using the set of 48
241 variables common to both NFBC1966 and DESIR. Next, we trained the data in the NFBC1966 and tested
242 in DESIR. We predicted only FI and FG levels using the top three performing algorithms: RF, BT and
243 SVR-L2Linear-L1L2 (**Methods**). We were able to predict FI and FG levels in the DESIR data with the
244 average R^2 values of 0.30 and 0.17, respectively (**Figure 4**, **Supplementary Table 12**). The values were
245 decreased as compared to those when trained and tested in the same data, i.e. NFBC1966 (R^2 for
246 FI=0.55, FG=0.41, **Supplementary Table 12**). RF and SVR-L2Linear-L1L2 produced a smaller change in
247 the predictions (FI: RF=0.18, SVR-L2Linear-L1L2=0.22; FG: RF=0.21, SVR-L2Linear-L1L2=0.19), while BT
248 was less stable in its performance (change in R^2 0.35 for FI, 0.32 for FG). Having a larger sample size in
249 the training data (**Methods**) produced less variable R^2 values than those resulting from the use of a
250 smaller training dataset in all models (**Figure 4A** vs. **4B**). While we still observed a drop in the R^2 values

251 for both FG and FI when tested in the external data, RF and SVR-L2Linear-L1L2 produced smaller
252 decreases than BT, as before (**Supplementary Table 12**).
253

254 Discussion

255

256 To our knowledge, this is the first multi-omics study implementing machine learning to predict
257 continuous glycaemic traits over time. We dissected the predictive value of methylation and
258 metabolic, including metabolomic, data from two time points for individual's glycaemic health. We
259 compared six machine learning approaches, while most previous studies have usually focussed on only
260 one selected approach. We detected the best predictive ability of our models for FI and FG levels out
261 of the five glycaemic traits tested, with raw or scored metabolic data as predictors and, BT, RF, or SVR-
262 L2Linear-L1L2 as the algorithms. We identified metabolic variables that drove the prediction of the
263 models. We showed that measures of adiposity are the most important contributors to glycaemic
264 health. We also found that methylation probes accounted for 4 and 3 percentage points of the variance
265 explained for FI (58%) and FG (45%), respectively. Finally, replication of the approach in an external
266 European descent dataset (DESIR) using a subset of variables common to both cohorts, suggested that
267 RF and SVR-L2Linear-L1L2 are more stable than BT in their performance.
268

269 Most of the published studies have targeted T2D onset prediction as a discrete value and rely on the
270 categorization of individuals based on diagnosis thresholds for HbA1c, FG, 2hGluc and random glucose.
271 The cut-off points for prediction analyses may vary across studies, and more generally, information
272 loss is rather large when data is categorised[28,29]. Continuous phenotypes, on the contrary, have the
273 potential to reflect the progressive onset of a disease without assuming a discontinuity in the
274 underlying phenomenon. In addition, focussing on the prediction of continuous glycaemic phenotypes
275 themselves allows removing them from the set of predictors for T2D and may reveal more modest
276 effects of other variables[24]. Indeed, FG[23–27] and 2hGluc[24,25] have been shown to be good predictors of

277 T2D. Our study indicated that out of the five glycaemic traits we used, FG and FI were best predictable.
278 This is expected as fasting values are tightly regulated. However, from the clinical practice point of
279 view regarding the prediction of developing T2D, especially FI measurements have less relevance as
280 compared to HbA1c measurements, for example. This suggests that future efforts should be directed
281 towards improving the prediction of the other glycaemic indices than FG and FI.
282
283 Our study leverages machine learning ability to perform variable selection independently of a pre-
284 filtering. To date, RLS (a variant of SVR-L2Linear algorithms)[27], J48-decision tree[25], and logistic
285 regression with regularization[23,26] have highlighted the importance of specific metabolites consistent
286 with our findings. Indeed, branched-chain amino acids (Leucine, Valine, Isoleucine)[25,26], HDL, VLDL,
287 glycerol, ApoA and Apo B, 3-hydroxybutyrate[26], aromatic amino acids (phenylalanine, tyrosine)[23,26] are
288 established as important predictors by machine learning algorithms, as also shown by our study.
289 Moreover, in this study, we report glycoprotein acetyls and acetoacetate as good predictors of
290 glycaemic trait levels. These markers have previously been associated with T2D[30–32]; however, for the
291 first time here, we show that they are not only associated, but are also predictors of glycaemic health.
292
293 In addition to specific metabolites, the machine learning algorithms assigned a high rank (first 25) to
294 several established metabolic health-associated methylation probes in the prediction of FI and FG
295 when collapsing metabolic predictors into scores but keeping methylation probes as such. The probes
296 included for instance those within the genes *CPT1A* and *SREBF1*, where the first, *CPT1* (Carnitine
297 palmitoyltransferase I) is involved in fatty acid metabolism (RefSeq, Jul 2008) , and the latter, *SREBF1*
298 (Sterol regulatory element-binding transcription factor 1) regulates genes required for glucose
299 metabolism as well as fatty acid and lipid production, and its expression is regulated by
300 insulin[33]. Previously, methylation at *CPT1A* and *SREBF1* has been associated with 2hIns[19], BMI[20], FG[18]
301 and T2D[18]. In our study, hypomethylation at *CPT1A* (cg17058475) at T2 predicted higher FI and FG
302 levels, whereas hypermethylation at *SREBF1* (cg11024682) at T1 predicted higher FI levels. The

303 predictive power of the methylation probes was modest, 0.04 out of 0.58 for FI and 0.03 out of 0.45
304 for FG. However, for both glycaemic traits the ranking of 15 methylation probes among the top 25
305 predictors when using scored metabolic data, sets the ground for larger studies of this kind, similarly
306 to the work that has been achieved through large-scale GWAS. A recent study aggregating information
307 over millions of genetic markers into a score showed that genetic risk scores for common diseases can
308 identify people at risk equivalent to monogenic mutations[34]. On that account, the findings from our
309 study encourage further exploration of methylation scores consisting of thousands or even millions of
310 probes in glycaemic trait level prediction.
311
312 Measures of body adiposity and those of obesity are established risk factors for T2D[35] and have a well-
313 known impact on glycaemic trait variability[36]. In all six machine learning approaches and within all data
314 combinations, we confirmed the high predictive value of BMI and WHR already 15 years beforehand.
315 Indeed, when we calculated the variance explained from linear regression with the top 25 predictors,
316 BMI and WHR accounted for the most part of it for both FI (0.40 out of 0.58) and FG (0.31 out of 0.45).
317 When the outcomes were adjusted for the measures of adiposity, FG and FI levels at T1 gained more
318 weight as predictors. These findings emphasize the importance of classical risk factors in T2D
319 prediction but also show that the tracking is relevant already 15 years beforehand. Taken together, it
320 is clear that classical risk factors will remain as valuable tools in the clinical practice for predicting
321 future glycaemic health. However, more detailed biomarkers, for example certain metabolites as
322 shown in the present study, genetic risk factors as shown recently[34] and possibly methylation markers
323 will open avenues for more precise prediction.
324
325 From the algorithm point of view, our analyses showed that the highest prediction performance was
326 achieved with BT, RF and SVR-L2Linear-L1L2 algorithms. This is consistent with the literature as BT and
327 RF have shown to perform well in the prediction studies of various types of data[37,38] . The SVR-L2Linear-
328 L1L2 (LIBLINEAR library in R) algorithm performed significantly better than the SVR-L2LinearL1

329 (kernelab library in R). This difference can be explained by the use of the L2-loss over the L1-loss in the
330 SVR-L2Linear-L1L2 in 78.5% of the cases (i.e. across all external cross-validations, all phenotypes and
331 all data type combinations). We further investigated whether the better performance was due to the
332 choice of the evaluation criterion, namely R^2 which evaluates the performance in squared terms,
333 similar to L2 loss, rather than in absolute terms, as do MAE and L1. Our analyses showed that SVR-
334 L2Linear-L1 performed worse, regardless of the evaluation criterion used. These observations highlight
335 the importance of loss function choice when using the SVR linear with L2-regularisation, independent
336 of the model performance evaluation criterion. The SVR-Polynomial and SVR-RBF showed slightly
337 lower predictions of the phenotypes, but there were no statistically significant differences when their
338 performances were compared to those of the top three algorithms.
339
340 Our analysis has some limitations that warrant discussion. First, the relatively small sample size (513
341 subjects) is a drawback for taking full advantage of machine learning prediction with high-dimensional
342 multi-omics data layers. However, our data is the largest to our knowledge featuring both longitudinal
343 data and a comprehensive set of multi-omics data. UK Biobank has expressed its plans to acquire
344 methylation data on its participants[39]. These data will be an important resource for future methylation
345 studies. Nevertheless, the data will be cross-sectional and will not allow investigation of changes in
346 methylation as the data used in the current study data does. Second, parameter tuning and drawing
347 of a threshold regarding variable importance in our machine learning models are not trivial. Longer
348 parameter tuning times might have resulted in more precise predictions and better performance of
349 some or all of the algorithms. Variable importance in turn will depend on the number of variables
350 resampled by the algorithms or the regularization parameters chosen. Overall, we restricted our
351 analyses to six machine learning algorithms in total. It was out of the scope of this study to explore
352 other potentially relevant algorithms, which will remain of future research interest. Third, regarding
353 the samples and the study design, the use of whole blood only for methylation markers, and the
354 relatively young age of the participants, 46 years old at the measurement time of the outcome

355 variables is a limitation. The latter might alternatively represent a positive feature, since blood is the
356 easiest tissue to obtain for any study, while the trend of deteriorating glycaemic health in younger
357 adults is growing in all human populations. Finally, our replication effort also has some limitations. We
358 were able to integrate only a small number of variables shared by both cohorts, and the time between
359 the measurements differed between the Finnish and French studies, as did the age of the participants
360 at recruitment. Despite these limitations the tested machine learning models showed promising
361 consistency between the two cohorts, and we would expect better performance would the
362 abovementioned limitations be addressed better.
363

364 ## Conclusions

365 With the use of six different machine learning algorithms, we have identified clinically relevant sets of
366 predictors of glycaemic traits from large multi-omics datasets and highlighted the potential of
367 methylation markers and longitudinal changes in prediction. In the future, we expect that
368 improvements in study sizes, methylation score computation, finer model tuning and replication in
369 more similar external datasets will improve predictive ability of our models for glycaemic traits and
370 will unveil novel prognostic omics biomarkers for T2D endophenotypes.
371

372 ## Methods

373 **Study Populations**

374 **NFBC1966**

375 Northern Finland Birth Cohort 1966 (NFBC1966) comprises participants from the two northernmost
376 provinces of Finland with expected dates of birth falling in 1966 (N=12,058 births)[40]. From the medical
377 examination at 31 (T1, N=6,007) and 46 years (T2, N=5,861) we included participants with
378 demographic, medication, epidemiological, blood biochemical, metabolomic and epigenetic

379 information available at both time points (N=626). Consent was obtained and the study was approved
380 by the ethical committees of the University of Oulu and Imperial College London (Approval:18IC4421).
381
382 *DESIR*
383 The longitudinal DESIR study (Data from an Epidemiological Study on the Insulin Resistance
384 syndrome) included 5,212 participants from the French general population. Clinical and biological
385 evaluations were performed at inclusion and after three, six, and nine years, as previously
386 described[41,42]. We used the data from 769 individuals aged from 30 to 65 years old at time of inclusion
387 (T1) and nine years after inclusion (T2). Written informed consent was obtained from all participants.
388 The study was approved by the Ethics Committee for the Protection of Subjects for Biomedical
389 Research of Bicêtre Hospital, France.
390
391 **Epidemiological, Blood Biochemical and Metabolomic Data in the NFBC1966**
392 Height, weight, waist and hip circumference, and systolic and diastolic blood pressure (SBP, DBP, each
393 measured in triplicate) were measured according to standard study protocols at the clinical
394 examinations at T1 and T2. We used the measured height and weight; however, if unavailable, data
395 from postal questionnaire were used. Body mass index (BMI) was calculated from height and weight
396 and waist-hip-ratio (WHR) from waist and hip measurements accordingly. The biochemical assays[43,44],
397 oral glucose[45], and HbA1c measurements[46] are detailed elsewhere. Metabolites were quantified by a
398 high-throughput serum nuclear magnetic resonance (NMR) platform[47–50]. Imputation of
399 epidemiological, biochemical and metabolomic variables was performed jointly with random forest
400 (MisForest in R[51]) (**Supplementary Methods**). Post imputation, for individuals with diagnosed T2D at
401 T2 (N=18), we corrected their potentially T2D medication-induced and thus artificially normal FG
402 values to 7 mmol/l, HbA1c values to 48 mmol/l (6.5%) and 2hGluc values to 11.1 mmol/l. Detailed
403 descriptions of all the exclusions and corrections are given in the **Supplementary Methods**. Finally, all
404 the predictor variables were normalised using inverse-normal transformation. The epidemiological,

405 blood biochemical and metabolomic variables used in the predictions are listed in **Supplementary**
406 **Table 1**.
407
408 **Epigenomic Data in NFBC1966**
409 DNA methylation was measured in whole blood from 807 randomly selected individuals after
410 overnight fasting. At T2, DNA methylation was measured for 758 selected subjects that attended the
411 clinical examination, completed the questionnaire and had DNA methylation data from the previous
412 clinical examination available. IlluminaInfiniumHumanMethylation450 Beadchip and EPIC arrays were
413 used at T1 and T2, respectively. Methylation data was quality controlled according to study protocol
414 (**Supplementary Material**) and pre-processed on genome build CGCh37/hg19. Imputation of
415 methylation data was performed with random forest (MisForest in R[51]) using the methylation residuals
416 corrected for sex, and blood cell type (**Supplementary Material**). We limited our analysis to the
417 methylation probes previously associated with seven phenotypes: 187 probes associated with BMI[20],
418 21 with FG[18], 11 with HbA1c[18] and 68 with T2D[18], one with 2hGluc[19], eight with FI[19], 21 with 2hIns[19]
419 (**Supplementary Table 2**).
420
421 **Epidemiological, Blood Biochemical and Metabolomic Data in DESIR**
422 A total of 48 predictor variables overlapped between NFBC1966 and DESIR data. These included sex,
423 measures of adiposity, biochemical data (triglycerides, total cholesterol, high and low-density
424 lipoprotein cholesterol (HDL-C and LDL-C) at T1 and T2, insulin and glucose at T1), and metabolomic
425 data (32 variables, 17 at T1 and 15 at T2). A full list of the included variables is given in **Supplementary**
426 **Table 3**. The data were imputed with the package MisForest in R[51] (missingness rate < 1%)
427 (**Supplementary Material**). The values of FG were set to 7 mmol/l if the individual had diagnosed T2D.
428 Finally, all the predictor variables were normalised using inverse-normal transformation.
429
430

431 **Individuals and Study Variables in the Machine Learning Models**

432 In total, 513 individuals in the NFBC1966 were included in the machine learning analysis. HbA1c,
433 2hGluc, 2hIns, FG, FI levels were used as continuous outcomes to predict. A total of 1,001 variables
434 from T1 and T2 were used as predictors in the NFBC1966. Metabolic predictors included:
435 epidemiological data - sex, measures of adiposity (BMI and waist-to-hip ratio), SBP and DBP,
436 biochemical data - ten blood measurements of triglycerides, total cholesterol, high and low-density
437 lipoprotein cholesterol (HDL-C and LDL-C), metabolomic data - 454 metabolites (228 at T1 and 226 at
438 T2) (**Supplementary Table 1**). The methylation dataset included 528 unique probes, including 264 at
439 T1 and 264 at T2 (**Supplementary Table 2**).

440

441 For the replication analysis we chose to predict only FG and FI, for which the predictions worked the
442 best in the NFBC1966 analyses. We included 48 variables common to both cohorts (**Supplementary**
443 **Table 3**). The DESIR Cohort did not encompass methylation data. Therefore, before testing the
444 prediction ability of our models in 769 individuals from DESIR, we could train the algorithms in the
445 NFBC1966 either with the 513 individuals as previously, or with all 3,056 individuals who had
446 metabolomics data available. This allowed us to test whether prediction in the external cohort was
447 improved by increasing the sample size of the training data.

448

449 **Predictor Combinations and Prediction Frameworks**

450 Metabolic (Mb) and Methylation (Mh) data were combined as their individual (or raw, denoted here
451 as R) values or transformed into scores (S) (**Supplementary Material**). Methylation scores were formed
452 according to the traits the probes have been previously associated with (**Supplementary Table 2)** and
453 metabolic scores according to specific categories (**Supplementary Table 4**). We refer to Mb-R/Mh-R
454 when the input data are all represented as individual values and to Mb-S/Mh-S when all the input data
455 are combined in scores. Sex was kept separate and included in the model in addition to the scored
456 variables. The following combinations were tested: Mb-R/ Mb-S/ Mh-R / Mh-S/ Mb-R + Mh-R/ Mb-R +

457 Mh-S/ Mb-S + Mh-R/ Mb-S + Mh-S (**Figure 1**). Methylation and Metabolic data were either adjusted
458 for BMI and waist-hip-ratio at T1 and T2, or kept unadjusted.
459
460 **Machine Learning Approaches**
461 Three machine learning methods were used for regression analysis: Boosted trees (BT), Random Forest
462 (RF) and Support Vector Regression (SVR) (**Supplementary Material**). SVR was implemented with
463 Linear Kernel with L2 regularization and with L1 and L1/L2 loss functions (SVR-L2Linear-L1, SVR-
464 L2Linear-L1L2, respectively), with Polynomial Kernel (SVR-Polynomial) and with Radial Basis function
465 Kernel (SVR-RBF) (**Figure 1**). For all the analyses, the packages kernelab, LIBLINEAR, RandomForest and
466 xgboost in R[51] were used with Caret as a wrapper.
467
468 **Optimization of the Machine Learning Algorithms**
469 Nested cross validation was implemented. The data set was split into a training (80%) and testing set
470 (20%) with a 5-fold cross validation. The performance of the machine learning models was estimated
471 on the testing set, while parameter tuning was implemented on the training set by splitting it further
472 into a 5-fold cross validation (nested). Random search method was used to find the model parameter
473 combination (**Supplementary Table 13**) which minimized the error of the model. The Root Mean
474 Square Error (RMSE) was used to assess model performance during training. Both Rsquared (R^2) and
475 RMSE were computed in the testing set to estimate performance. For additional checks we used the
476 Mean Absolute Error (MAE) to assess model performance.
477
478 **Variable Importance in the Machine Learning Models**
479 In BT, the information gain was used as a measure of importance. Gain is based on the decrease in
480 entropy after a dataset is split on a feature j at a branch of the tree. RF variables were ranked with the
481 Increase in Mean Square Error (MSE). It estimates the increase of prediction error when the values of

482 the feature j are randomly permuted. For SVR, each feature is evaluated based on its independent
483 association with the outcome. The slope of the regression is used to rank the features.
484
485 **Statistical Analysis for Comparing Model Performance**
486 The performance of each model was computed as the average R^2 over the 5 testing folds of the cross
487 validation. In the Results section, we report the R^2 pooled for the six machine learning algorithms.
488 Comparison of the models was performed with a one-way ANOVA and post-hoc Tukey honest
489 significance test (HSD) test. We use *P*-value<0.05 to denote statistical significance.
490
491 **Association Analysis to Obtain Effect Size, Direction and Variance Explained**
492 We performed linear regression analyses between the best predicted outcomes (FG and FI) and each
493 of the top 25 predictors suggested by the machine learning models to assess the effect sizes and
494 directions of the associations. The analyses were conducted in R[51]. We report betas with their standard
495 errors and related *P*-values. Additionally, we performed linear regression analyses for ln(FI)/FG by
496 including all the top 25 predictors in the same model and then by removing metabolites/ methylation
497 markers from the set of predictors to evaluate the variance explained and the contribution of
498 metabolites/methylation markers in it. We report the adjusted R^2 from these analyses.
499
500 **Replication analysis**
501 For the replication analysis we used the three most consistently performing machine learning
502 approaches: BT, RF, and SVR-L2Linear-L1L2. First, we estimated the performance of the algorithms in
503 the NFBC1966 using a restricted set of 48 predictors. We selected the 48 input variables as overlapping
504 with our validation cohort variables. This performance estimation was done by splitting the NFBC1966
505 dataset of 513 individuals into 80% for training and 20% for testing (nested cross validation for tuning
506 as described above). Second, we "re-trained" the model with the maximum number of individuals in
507 the NFBC1966, i.e. 513 individuals which represent 100% of the previous dataset with methylation and

508 metabolomics data, or all 3,056 individuals with metabolomics data only. Then we tested for the ability
509 of this model to predict accurately glycaemic traits when given an independent population (DESIR)
510 with the same input variables. Practically, we used either 513 or 3,056 individuals from the NFBC1966
511 for training and used the resulting models to predict FI and FG in 769 individuals of the DESIR cohort.
512 Performance was evaluated with R2 as described above.
513

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Author contributions

IP conceived the study idea. IP, MK, LP, HD and ZB defined the phenotypes and quality control criteria. LP, HD, MDA, MW and MK performed the analyses. LY, BB, RR, SS, MAK, PF and MRJ provided the data. LP, MK and IP formed the central writing group. All authors participated in the critical revision of the manuscript and approved the final version.

Competing Interests

The authors declare no competing interests.

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671
672

673 **Figure legends**

674

675 **Figure 1. Experimental set-up for Machine learning analysis.** We applied machine learning to multi-
676 omics data based on blood samples and data collections from the Northern Finland Birth Cohort 1966
677 at 31 and 46 years. Fasting glucose/insulin (FG/FI), glycated haemoglobin (HbA1c) and 2-hour
678 glucose/insulin (2hGlu/2hIns) phenotypes at T2 were predicted in 513 individuals using up to 991
679 variables from T1 and T2: Body-mass-index (BMI), waist-hip-ratio, systolic and diastolic blood pressure
680 (SBP and DBP), sex; 10 blood plasma measurements; 453 NMR-based metabolites; 528 methylation
681 probes established for BMI, FG, FI, HbA1c, 2hGlu, 2hIns or Type 2 diabetes. Six machine learning
682 approaches were used: random forest, boosted trees and support vector regression (SVR) with the
683 kernels of linear function with L2 regularization and L1 loss function (L2Linear-L1), linear with L2
684 regularization and L1/L2 loss function (L2Linear-L1L2), polynomial and radial basis function (RBF).

685

686 **Figure 2. Performance as measured in R^2 of the different machine learning models.** A. Unadjusted for
687 measurements of adiposity (Waist-to-hip-ratio and Body mass index) at T1 and T2; B. Adjusted for
688 measurements of adiposity (Waist-to-hip-ratio and Body mass index) at T1 and T2. Training of the
689 algorithm was performed with a nested cross validation (5-folds outer, and 5-folds inner cross
690 validation) and the R^2 of 5 outer testing folds is displayed for each machine learning model. Metabolic
691 predictors include epidemiological, biochemical and metabolomic data. SVR: Support Vector
692 Regression with the kernels of linear function with L2 regularization and L1 loss function (L2Linear-L1),
693 linear with L2 regularization and L1/L2 loss function (L2Linear-L1L2), polynomial and radial basis
694 function (RBF).

695

696 **Figure 3. Variable Importance for fasting glucose and fasting insulin prediction from two different**
697 **combinations of predictor data.** For each Machine learning method, the normalized variable
698 importance over five outer fold of cross validation was averaged into the "Variable-Model-Importance"

699 (var.mod.Imp). Then for each of the six machine learning models, the variables were ranked based on
700 the var.mod.Imp. The rank was averaged over the six models to obtain the "mean variable rank". The
701 latter was used to select top 25 variables for display. For these variables, we display the variable
702 importance after (1) weighting the var.mod.Imp by the R^2 obtained for each of the individual machine
703 learning algorithms (2) averaging variable importance across the six machine learning models.
704 FI: Fasting Insulin; FG: Fasting Glucose; RF: random forest; BT: boosted trees, SVR: support vector
705 regression models with the kernels of linear function with L2 regularization and L1 loss function
706 (L2Linear-L1), linear with L2 regularization and L1/L2 loss function (L2Linear-L1L2), polynomial and
707 radial basis function (RBF). Metabolic predictors include epidemiological data, biochemical data and
708 metabolomic data. T2: 46 years old, T1: 31 years old. BMI: Body Mass Index according to clinical
709 examination, postal questionnaire if missing; WHR: Waist-to-hip ratio. Metabolite name descriptions
710 are provided in **Supplementary Table 1**.
711
712 **Figure 4. Performance as measured in R^2 of the different machine learning models with 48 variables**
713 **shared between NFBC1966 and DESIR.** A. Using NFBC1966 data with individuals having methylation
714 data (N=513) and all available DESIR individuals (N=769). B. Using NFBC1966 data with all available
715 individuals (N=3,056) and all available DESIR individuals (N=769). Training of the algorithm was
716 performed with a nested cross validation (5-folds outer, and 5-folds inner cross validation) and the R^2
717 of 5 outer testing folds is displayed for each machine learning model. Metabolic predictors include
718 epidemiological, biochemical and metabolomic data. SVR: Support Vector Regression with the kernel
719 of linear function with L2 regularization and L1/L2 loss function (L2Linear-L1L2).
720

721 **Tables**

722 **Table 1. Effect of the input dataset on the prediction performance of the five glycaemic traits.**

Comparison	Model	Input dataset				Number of outcome variables for which the model (A or B) performs the best
		Mb-R	Mb-S	Mh-R	Mh-S	
1	A	x				5/5, $P<2.3\times10^{-10}$
	B			x		0/5
2	A	x				5/5, $P<1.8\times10^{-11}$
	B				x	0/5
3	A		x			5/5, $P<1.7\times10^{-9}$
	B			x		0/5
4	A		x			5/5, $P<6.0\times10^{-14}$
	B				x	0/5
5	A	x				2/5, $P<0.04$ (equal performance for 3/5)
	B	x		x		0/5
6	A	x				0/5 (equal performance for all)
	B	x			x	0/5
7	A		x			0/5 (equal performance for all)
	B	x				0/5
8	A			x		1/5, $P<1.6\times10^{-4}$ (equal performance for 4/5)
	B				x	0/5
9	A		x		x	4/5, $P<0.02$ (equal performance for 1/5)
	B		x	x		0/5

723

724 Selected comparisons of models in pairs are displayed to illustrate different scenarios. 1-4) Comparison
725 between models with metabolic or methylation data only; 5-6) The effect of combining two data
726 types.; 7-8) The effect of variable transformations into scores; 9) The decrease in performance of the
727 models upon inclusion of a large number of predictors. Mb-R: Metabolic Raw Variables, Mb-S:
728 Metabolic Scored Variables, Mh-R: Methylation Raw Variables, Mh-S: Methylation Scored Variables.
729 Metabolic predictors include epidemiological data, biochemical data and metabolomic data. FG/FI:
730 Fasting glucose/insulin; HbA1c: glycated haemoglobin; 2hGlu/2hIns: 2-hour glucose/insulin.
731 The performance of all machine learning algorithms upon inclusion of different datatypes was
732 evaluated. For a given phenotype (FG, FI, HbA1c, 2hGlu or 2hIns), the effect of an input dataset was
733 assessed. Column 4 shows which model performed the best, and the number of outcomes for which
734 this pattern is observed. The *P*-value is the maximum *P*-value observed when comparing model A
735 against model B for each of the five outcomes. Models were compared with Tukey HSD test following

a one-way ANOVA. To test the effect of a given dataset, we run all six machine learning algorithms in a nested cross validation framework (5 outer, 5 inner folds), thereby each group compared included six (machine learning algorithms) x five (testing errors) = 30 R^2 measures.

740 **Table 2. Linear regression analysis results for ln(FI)/FG and the top 25 predictors.**

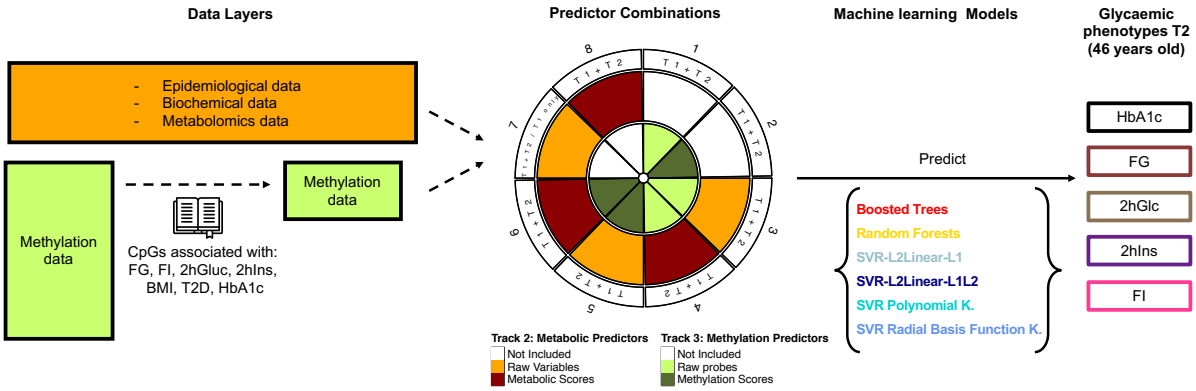
Mb-R + Mh-R	beta	SE	*P*-value	Mb-S + Mh-R	beta	SE	*P*-value
ln(FI)				**ln(FI)**			
T2_BMI	0.08	0.00	3.29×10^{-55}	T2_MeasuresOfAdiposity	0.08	0.00	5.75×10^{-56}
T2_Large_HDL_FreeChol_%	-0.11	0.01	1.48×10^{-40}	T2_BRACA	3.76	0.29	1.17×10^{-32}
T2_GlycoproteinAcetyls	1.32	0.10	5.88×10^{-37}	T1_MeasuresOfAdiposity	0.07	0.01	7.39×10^{-26}
T2_TrigyceridestoPhosphoglycerides	1.13	0.09	8.73×10^{-35}	T2_BloodProteins	1.32	0.10	3.36×10^{-37}
T2_WHR	3.79	0.27	3.12×10^{-38}	T2_Carbohydrates	0.73	0.06	1.62×10^{-26}
T2_Phenylalanine	29.08	2.32	1.62×10^{-31}	T2_cg00574958	-9.28	1.48	8.62×10^{-10}
T2_Large_HDL_FreeChol	-4.91	0.39	1.03×10^{-31}	T2_OtherAminoAcids	0.66	0.18	3.42×10^{-4}
T2_Large_HDL_TotChol_%	-0.04	0.00	2.04×10^{-34}	T2_cg06898549	3.01	0.63	2.54×10^{-6}
T2_Isoleucine	15.32	1.23	2.52×10^{-31}	T2_cg17058475	-4.67	1.12	3.45×10^{-5}
T2_Large_HDL_TotChol	-1.14	0.09	4.61×10^{-30}	T1_CarbohydratesAndInsulin	0.10	0.02	1.68×10^{-9}
T2_Leucine	13.13	1.07	1.09×10^{-30}	T2_cg08309687	-3.51	0.68	3.24×10^{-7}
T1_FI	0.80	0.07	1.19×10^{-27}	T2_KetonBodies	-0.70	0.29	1.52×10^{-2}
T2_Large_HDL_CholEsters	-1.47	0.12	1.86×10^{-29}	T2_cg10927968	3.01	0.64	3.23×10^{-6}
T2_Large_VLDL_PhosphoLipids	4.59	0.41	1.76×10^{-26}	T1_cg08309687	-2.44	0.63	1.10×10^{-4}
T2_Glycerol	11.03	0.94	3.94×10^{-28}	T2_Lipoparticules	0.00	0.00	4.99×10^{-9}
T2_VLDL_Trigycerides	0.40	0.03	6.03×10^{-28}	T1_cg26361535	1.76	0.70	1.29×10^{-2}
T2_Lactate	0.74	0.07	2.34×10^{-25}	T2_cg25217710	4.83	1.27	1.51×10^{-4}
T2_Tyrosine	20.79	1.87	5.98×10^{-26}	T1_cg11832534	3.30	1.13	3.54×10^{-3}
T2_XL_HDL_PhosphoLipids	-1.30	0.11	1.81×10^{-28}	T1_cg11024682	3.92	1.02	1.33×10^{-4}
T2_Large_HDL_Trigycerides_%	0.10	0.01	3.53×10^{-26}	T1_cg09469355	-3.98	0.93	2.11×10^{-5}
T2_XL_VLDL_Trigycerides	4.04	0.38	3.47×10^{-24}	T1_cg07728579	1.87	1.00	6.18×10^{-2}
T2_Valine	5.83	0.54	1.48×10^{-24}	T2_cg25096107	-3.36	1.40	1.68×10^{-2}
T2_Large_HDL_Particules	-375767.70	31515.32	4.35×10^{-29}	T2_cg26804423	4.02	1.10	2.96×10^{-4}
T2_XL_HDL_PhosphoLipids_%	-0.02	0.00	2.03×10^{-26}	T2_cg04524040	-3.21	0.89	3.59×10^{-4}
T2_Medium_VLDL_TotChol	2.45	0.22	6.10×10^{-26}	T1_BloodProteins	0.39	0.08	1.52×10^{-6}
FG				**FG**			

T2_Valine	7.43	0.56	7.64×10^{-35}	T2_BRACA	4.52	0.31	2.79×10^{-41}
T2_Leucine	15.38	1.12	1.26×10^{-36}	T2_MeasuresOfAdiposity	0.06	0.01	2.57×10^{-28}
T2_WHR	3.73	0.30	4.59×10^{-31}	T1_MeasuresOfAdiposity	0.06	0.01	2.56×10^{-14}
T2_BMI	0.06	0.01	8.60×10^{-28}	T2_BloodProteins	1.05	0.11	1.42×10^{-19}
T2_Isoleucine	16.56	1.33	2.53×10^{-31}	Sex	-0.41	0.05	1.37×10^{-14}
T2_Tyrosine	20.74	2.05	5.73×10^{-22}	T2_OtherAminoAcids	0.96	0.19	1.08×10^{-6}
T2_SystolicBloodPressure	0.01	0.00	2.82×10^{-20}	T1_BRACA	1.88	0.31	4.10×10^{-9}
T1_WHR	2.80	0.30	7.01×10^{-19}	T1_CarbohydratesAndInsulin	0.13	0.02	4.46×10^{-13}
T1_FG	0.48	0.05	1.85×10^{-18}	T2_cg11080651	-8.53	1.94	1.39×10^{-5}
T2_Large_HDL_PhospholLipids_%	0.05	0.01	1.04×10^{-19}	T2_cg19695507	4.06	1.13	3.57×10^{-4}
T2_Large_HDL_FreeChol_%	-0.09	0.01	2.80×10^{-20}	T2_cg19693031	-2.79	0.73	1.53×10^{-4}
T2_Small_HDL_Trigycerides	10.63	1.52	7.58×10^{-12}	T2_Lipoparticules	0.00	0.00	9.06×10^{-8}
T2_DiastolicBloodPressure	0.02	0.00	1.00×10^{-16}	T2_cg26403843	2.07	0.60	6.44×10^{-4}
T2_Medium_VLDL_Trigycerides_%	0.03	0.00	7.61×10^{-12}	T2_cg25217710	4.17	1.38	2.58×10^{-3}
T2_TrigyceridestoPhosphoglycerides	0.88	0.10	1.77×10^{-17}	T2_cg09777883	2.20	1.09	4.32×10^{-2}
T2_Glycerol	8.74	1.08	4.71×10^{-15}	T1_cg04816311	1.75	0.67	8.93×10^{-3}
T2_Alanine	2.89	0.35	8.41×10^{-16}	T2_Carbohydrates	0.51	0.07	2.37×10^{-11}
T2_XL_HDL_Particules	-768070.10	98342.58	3.27×10^{-14}	T1_cg03497652	1.96	0.70	5.47×10^{-3}
T2_IDL_FreeChol_%	-0.18	0.02	2.22×10^{-15}	T1_cg11183227	3.85	1.10	5.01×10^{-4}
T2_Large_HDL_CholEsters	-1.15	0.14	3.18×10^{-15}	T1_cg23906191	4.99	1.89	8.39×10^{-3}
T2_XL_HDL_CholEsters_%	0.02	0.00	7.27×10^{-12}	T1_cg00574958	-4.21	1.64	1.06×10^{-2}
T2_Large_HDL_TotChol	-0.89	0.11	9.31×10^{-16}	T1_cg02059849	5.61	1.36	4.20×10^{-5}
T2_Medium_VLDL_CholEsters_%	-0.04	0.01	8.94×10^{-13}	T2_cg00634542	2.90	1.30	2.65×10^{-2}
Sex	-0.41	0.05	1.37×10^{-14}	T2_cg17058475	-3.86	1.22	1.60×10^{-3}
T2_Small_VLDL_Trigycerides_%	0.03	0.00	1.58×10^{-12}	T2_cg06898549	2.47	0.69	3.89×10^{-4}

The models were fitted for each predictor separately. Left panel shows the results when the predictors were in their raw format (Metabolic-Raw, Mb-R + Methylation-Raw, Mh-R) and right panel shows when the metabolic predictors were transformed into scores but methylation data kept in raw format (Metabolic-Score, Mb-S + Methylation-Raw, Mh-R).

744 **Figures**

745 **Figure 1.**



746

747 **Figure 2.**

748

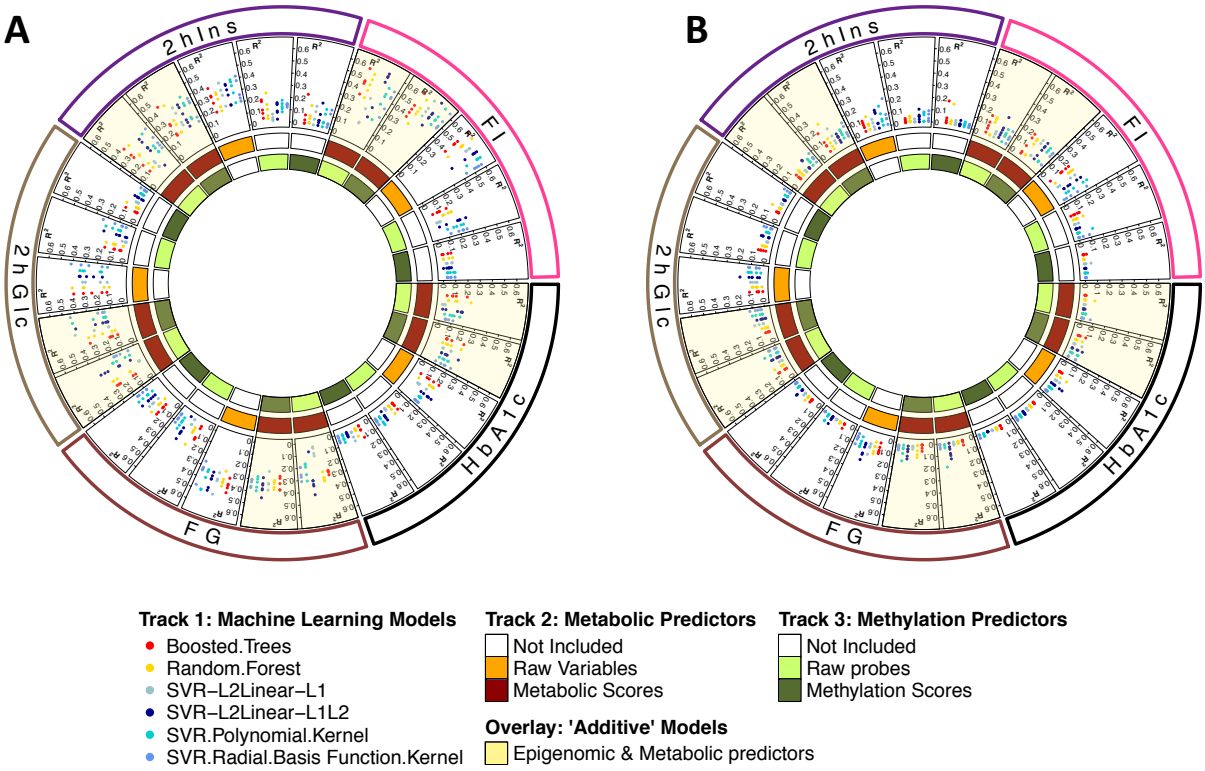


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Figure 3.

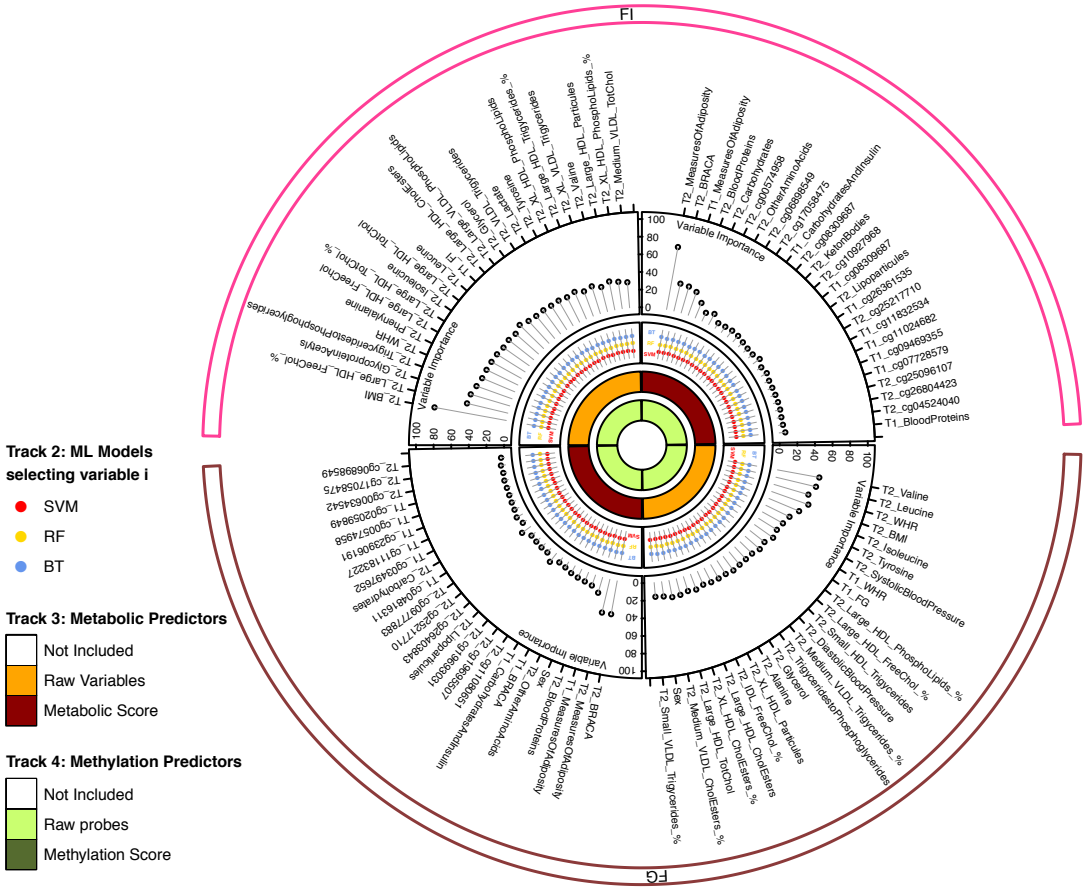
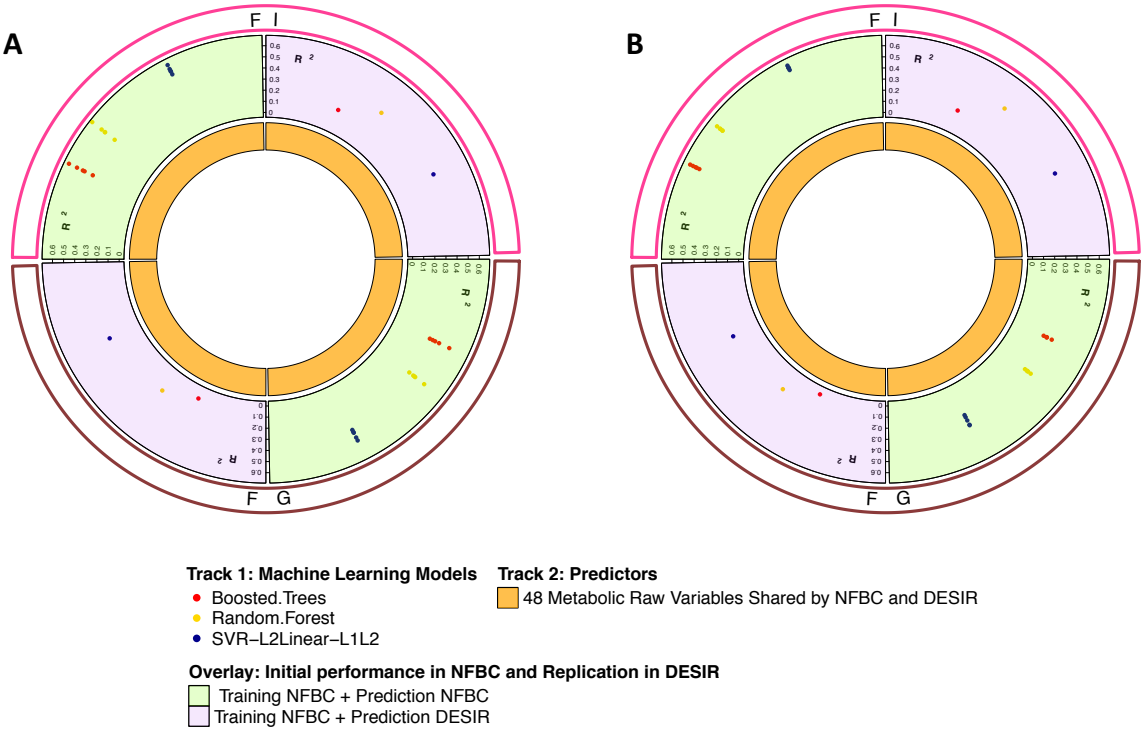
Figure 4.