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Baseline phenotypes with preserved β-cell function and high insulin concentrations have the best improvements in glucose tolerance after weight loss: results from the prospective DEXLIFE and EGIR-RISC studies

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ABSTRACT

Background: Weight loss and lifestyle intervention improve glucose tolerance delaying the onset of type 2 diabetes (T2D), but individual responses are highly variable. Determining the predictive factors linked to the beneficial effects of weight loss on glucose tolerance could provide tools for individualized prevention plans. Thus, the aim was to investigate the relationship between pre-intervention values of insulin sensitivity and secretion and the improvement in glucose metabolism after weight loss.

Methods: In the DEXLIFE cohort (373 individuals at high risk of T2D, assigned 3:1 to a 12-week lifestyle intervention or a control arm, Trial Registration: ISRCTN66987085), K-means clustering and logistic regression analysis were performed based on pre-intervention indices of insulin sensitivity, insulin secretion (AUC-I), and glucose-stimulated insulin response (ratio of incremental areas of insulin and glucose, iAUC I/G). The response to the intervention was evaluated in terms of reduction of OGTT-glucose concentration. Clusters' validation was done in the prospective EGIR-RISC cohort (n = 1538).

Results: Four replicable clusters with different glycemic and metabolomic profiles were identified. Individuals had similar weight loss, but improvement in glycemic profile and β-cell function was different among clusters, highly depending on pre-intervention insulin response to OGTT. Pre-intervention high insulin response was associated with the best improvement in AUC-G, while clusters with low AUC-I and iAUC I/G showed no beneficial effect of weight loss on glucose control, as also confirmed by the logistic regression model.

Conclusions: Individuals with preserved β-cell function and high insulin concentrations at baseline have the best improvement in glucose tolerance after weight loss.

Abbreviations: AUC-G, area under the curve of glucose; AUC-I, area under the curve of insulin; AUROC, area under receiving operating characteristic curve; BCAA, brain-chain amino acids; BMI, body mass index; CI, confidence intervals; CT, control group; FDR, false discovery rate; HDL, high-density lipoprotein; Hep-IR, hepatic insulin resistance; HOMA, homeostasis model assessment; i-AUC I/G, ratio of the incremental area under the curve of insulin over glucose; IGT, impaired glucose tolerance; IFG, impaired fasting glucose; ISI, insulin sensitivity index; KNN, K-nearest neighbors; LDL, low-density lipoprotein; LGPC, linoleoyl-glycero-phosphocholine or LysoPC (18:2); LI, lifestyle intervention group; NG, normal glucose; OGTT, oral glucose tolerance test; T2D, type 2 diabetes; UHPLC-MS/MS, Ultra-high performance liquid chromatography tandem mass spectrometry; VO_2max, maximal oxygen consumption.

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1. Introduction

The global prevalence of type 2 diabetes (T2D) is rapidly increasing and was estimated to be 9.3 % (463 million people) in 2019, but it will almost double in 2045 (700 million people) [1]. For the healthcare system, the burden of T2D is a major concern and effective measures to prevent and/or delay the onset of the disease are especially needed.

The main factors associated with the development of T2D are obesity, insulin resistance and impaired β-cell response to the increase in glucose concentrations [2]. The United Kingdom Prospective Diabetes Study and then other studies have shown that β-cell function estimated by the homeostatic model assessment (HOMA-B) was reduced by 40–50 % at time of diagnosis [3]. A β-cell reduction was also confirmed when measured during OGTT by the disposition index, either using 120 minutes [4,5] or 30 minutes [6] glucose and insulin profiles. As previously shown, disposition index is already reduced by 80 % in subjects with impaired (IGT) compared to normal glucose tolerance [4] and it is also predictive of the development of T2D over 10 years [5,6].

To delay and prevent the onset of T2D, the American Diabetes Association guidelines suggest lifestyle intervention with a combination of dietary advice and physical exercise [7]. The Diabetes Prevention Program [8,9] and the Finnish Diabetes Prevention Study [10,11] demonstrated how intensive lifestyle intervention and body weight loss could reduce the risk of incident T2D by up to 58 % over 3 years. Moreover, high intake of fibers and low saturated fat is advised to improve cardiometabolic risk [12].

Although body weight loss and lifestyle modifications lead in general to significant improvements in insulin sensitivity, metabolic flexibility, and lipid metabolism, the response to lifestyle intervention in terms of enhancement of glucose tolerance usually has a large inter-individual variability [13] and the factors that influence it are still largely unknown. Differences in prediabetes phenotypes and their underlying physiology have been proposed as possible contributors to the observed heterogeneity and evidence suggests that lifestyle programs for T2D prevention are more effective in subjects with IGT than in those with isolated impaired fasting glucose (IFG) [14]. Moreover, β-cell function, disposition index and insulin sensitivity measured during OGTT have been also previously associated with the risk of T2D [5,6] and the variability of phenotype response [13,15,16]. Thus, we hypothesize that the observed heterogeneity in the improvement of glucose tolerance following lifestyle intervention and/or weight loss is associated with pre-intervention low levels of insulin sensitivity and secretion.

We tested our hypothesis in the DEXLIFE (Diet and Exercise for Life) project that was designed to identify, in participants at high risk for T2D, novel diagnostic and predictive biomarkers that characterize the variance in response to a lifestyle intervention, based on personalized dietary advice and supervised physical activity [17]. The first analysis of this study reported that traditional phenotypic characteristics and physiological parameters (e.g., glucose tolerance, anthropometric characteristics, and aerobic fitness) were not sufficient to predict inter-individual variability in glucose tolerance post intervention [18]. Thus, we investigated if pre-intervention indices of insulin sensitivity and secretion derived from the OGTT (Matsuda index, ISI; OGTT-insulin response, AUC-I; β-cell response to glucose load, iAUC I/G) [19] can predict the improvement in glucose tolerance after weight loss following lifestyle intervention. We used machine learning to analyze the results of the DEXLIFE study to (i) identify pre-invention clusters of individuals and test how they responded to the intervention, and (ii) train a classification model to predict such response. To reinforce and validate our findings we tested the clustering in an external cohort, the EGIR-RISC 3-year study [20]. The result of this study will help identify which subjects will benefit most from the lifestyle and weight loss programs in terms of improved glucose metabolism.

2. Materials and methods

2.1. Study cohort

We used data from two cohorts: the DEXLIFE and the EGIR-RISC cohorts.

In the DEXLIFE project, a clinical trial was performed in the Dublin center to assess the impact of 12-week lifestyle intervention program in adults at high risk of developing T2D [17,18] (trial registration: ISRCTN66987085). Briefly, 373 subjects were enrolled and assigned to the lifestyle intervention group (briefly LI, n = 285) or the control group (CT, n = 88) with a ratio of 3:1 (see CONSORT flow diagram, Fig. S1), as described in reference [17].

The control group was given printed materials with general information about lifestyle and diabetes risk during an initial one-on-one meeting lasting about 15 min and was suggested to reduce weight if overweight/obese and increase physical activity levels [17]. The lifestyle intervention arm of the DEXLIFE study consisted of a combination of dietary advice [17] and supervised exercise training program for 12-weeks. All subjects were asked to fill a 3 day food diary to assess their dietary intake and then meet individually with a dietician to review the diary, identify unhealthy food choices and develop a plan to modify these choices by replacing unhealthy components of the diet with healthy alternatives, as previously reported [17]. A reduction in total calorie intake by 600 kcal/day has been recommended for subjects with obesity/overweight to achieve weight loss. Moreover, all subjects were asked to reduce to <10 % the saturated fat intake and increase the dietary fibers intake above 15 g/1000 kcal, irrespective of whether weight loss was a requirement or not. Additional information about the monitoring of the adherence to the intervention and the use of medications are reported in the Supplementary Material.

DEXLIFE inclusion criteria were: being without a previous diagnosis of diabetes, inactive (<150 min of physical activity per week) and showing at least one of the following risks factors: *i)* impaired fasting plasma glucose (levels ≥5.6 to <7 mmol/L), *ii)* impaired glucose tolerance (2 h plasma glucose levels ≥7.8 to <11.1 mmol/L following an oral glucose tolerance test), *iii)* normal glucose tolerance with a FINDRISC score > 12 (1 in 6 chance of developing T2D in the next 10 years) [10]. The protocol was approved by the Research Ethics Committee at Dublin City University (DCUREC/2012/080) and all subjects provided written informed consent [17]. A first analysis of the trial only explored the response of those that were in the intervention group [18] and did not include analysis of the control group.

The EGIR-RISC study was a multicenter observational study with a prospective 3-year follow-up [20,21] that examined the relationship between insulin sensitivity and cardiovascular disease in healthy people (n = 1538), i.e., individuals without hypertension or diabetes with OGTT performed at the time of enrollment and after 3 years. Participants were recruited in fourteen EU-countries, according to the previously published inclusion/exclusion criteria [20,21]. All subjects also filled a lifestyle questionnaire at baseline and follow-up [20]. The protocol was approved by the local ethics committee in each recruiting center and all subjects provided written informed consent [20].

2.2. Measurements and calculations

In the DEXLIFE intervention study, subjects were profiled pre and post intervention for: *i)* total body fat and fat-free mass, quantified by Dual X-ray absorptiometry; *ii)* subcutaneous and visceral fat depth (cm) was measured by ultrasonography; *iii)* measurement of maximal oxygen consumption (VO_2max); *iv)* glucose tolerance by a standard 75 g 3-h Oral Glucose Tolerance Test (OGTT) performed in the morning after an overnight fast; *v)* measurement of fasting serum concentrations of insulin, glucose, triglycerides, total and HDL-cholesterol.

In EGIR-RISC cohort, baseline measurements of glucose tolerance and insulin sensitivity were gathered by the 2-h 75 g OGTT and repeated

after 3 years.

Indices of insulin sensitivity/resistance like Matsuda index (ISI) and hepatic insulin resistance (Hep-IR) were calculated on the 2 h-OGTT as previously described [19]. Insulin response during OGTT was evaluated by the area under the curve of insulin during the 2 h-OGTT (AUC-I), using the trapezoidal rule. Disposition index was calculated as the ratio of incremental area under the curve of insulin to glucose (iAUC I/G) times the ISI as previously described [19].

Table 1
Anthropometric and clinical characteristics of the DEXLIFE study participants at baseline (n = 373) and after 12 weeks (n = 335).

	PRE			POST			LOG2 POST/PRE	
	N	**CT**	**LI**	**N**	**CT**	**LI**	**CT**	**LI**
Sex (M/F)	373	44/44	142/143	335	39/39	128/129	–	–
Age	373	54 [49;65.25]	53 [48;63]	335	54 [49;65]	53 [48;63]	–	–
Sys BP (mm/Hg)	359	132 [124.75;142]	132 [125;144]	314	128 [120;136.5]	129 [120;140]*	−0.05 [−0.13;0.02]	−0.03 [−0.16;0.07]
Dia BP (mm/Hg)	358	86 [80;90]	82 [80;90]	315	80 [74;88]	80 [72;82]*	−0.07 [−0.17;0.07]	−0.07 [−0.18;0.04]
BMI (kg/m^2)	372	30.56 [28.42;34.14]	30.76 [27.82;33.95]	324	30.67 [27.71;33.75]	28.54 [26.51;32.14]*°	0 [−0.02;0.02]	−0.06 [−0.1;-0.02] °
Weight (kg)	372	89.6 [78.25;101.6]	88.9 [76.5;102.3]	324	90 [77.18;101.85]	84.9 [73.66;95.2]*°	0 [−0.02;0.02]	−0.06 [−0.1;-0.02] °
VO_2Max (ml/kg min)	372	27.23 [23.17;33.39]	28.1 [23.6;33.63]	322	28.44 [23.78;33.73]	31.35 [25.42;37.49]*°	0 [−0.13;0.11]	0.12 [−0.01;0.27] °
Fat (kg)	373	37.27 [32.43;44.85]	38.37 [31.83;44.75]	324	37.15 [32.98;44.12]	34.81 [29.16;42.55]*	0.01 [−0.03;0.04]	−0.07 [−0.13;-0.01] °
Subcutaneous Fat (cm)	316	3.1 [2.2;3.89]	2.68 [2.04;3.55]	277	2.58 [1.93;3.58]	2.31 [1.77;3.06]*°	−0.08 [−0.35;0.16]	−0.19 [−0.44;0.04]
Visceral Fat (cm)	302	7.4 [6.07;8.73]	7.15 [5.76;8.58]	268	6.66 [5.8;8.93]	6.1 [4.75;7.37]*°	0 [−0.19;0.22]	−0.27 [−0.53;0.02] °
Glucose 0′ (mmol/l)	356	5.57 [5.32;5.98]	5.8 [5.34;6.2]	329	5.64 [5.21;5.96]	5.61 [5.23;6.02]*	−0.02 [−0.09;0.08]	−0.04 [−0.12;0.04]
Glucose 30′ (mmol/l)	353	8.89 [7.76;10.14]	9.47 [8.54;10.75]	325	8.99 [8;9.88]	9.28 [8.35;10.41]°	0.01 [−0.12;0.16]	−0.03 [−0.19;0.13]
Glucose 60′ (mmol/l)	351	8.92 [7.04;10.72]	9.31 [7.74;11.12]	323	9.16 [7.21;10.78]	9.15 [6.99;10.99]	0.02 [−0.14;0.3]	−0.05 [−0.29;0.11] °
Glucose 90′ (mmol/l)	353	6.98 [5.66;9.25]	7.38 [5.95;9.59]	321	6.9 [5.95;8.59]	7.3 [5.79;8.95]	0.06 [−0.2;0.25]	−0.11 [−0.34;0.13] °
Glucose 120′ (mmol/l)	350	6.15 [5.24;7.28]	6.25 [5.35;7.67]	326	6.05 [5.28;7.05]	6.13 [4.9;7.42]	0.02 [−0.15;0.2]	−0.13 [−0.35;0.13] °
Glucose 180′ (mmol/l)	335	4.61 [3.99;5.44]	4.65 [3.97;5.51]	306	4.48 [3.82;5.27]	4.54 [3.87;5.25]	0.01 [−0.23;0.09]	−0.03 [−0.27;0.14]
Insulin 0′ (pmol/l)	362	79.01 [57.02;109.4]	79.37 [51.4;121.43]	328	73.77 [54.35;108.9]	63.9 [40.85;91.38]*°	−0.01 [−0.36;0.38]	−0.23 [−0.62;0.12] °
Insulin 30′ (pmol/l)	359	512.2 [382.3;783.1]	508.9 [372.58;797.9]	325	450.3 [344.85;689.05]	444.2 [303.05;697.85]*	−0.09 [−0.43;0.19]	−0.17 [−0.6;0.21]
Insulin 60′ (pmol/l)	356	686.1 [412.35;934.2]	695.4 [463.5;1115]	325	621.05 [467.2;930.88]	553.5 [384;774.5]*°	0.11 [−0.25;0.43]	−0.29 [−0.7;0.12] °
Insulin 90′ (pmol/l)	360	532.4 [334.05;842.9]	606.5 [349.2;1034]	322	556.4 [357.38;857.93]	437.5 [275.63;682]*°	0.07 [−0.36;0.54]	−0.34 [−0.89;0.1] °
Insulin 120′ (pmol/l)	361	414.2 [227.4;733.9]	437.25 [256.95;753.93]	326	381.3 [261.58;658.83]	303.7 [182.2;499.83]*°	0.01 [−0.47;0.51]	−0.38 [−1.14;0.1] °
Insulin 180′ (pmol/l)	355	111.7 [75.51;217.3]	126.1 [71.94;238.1]	318	123.2 [70;218.55]	81.18 [47.92;157.4]*°	0.02 [−0.44;0.47]	−0.39 [−1.11;0.05] °
ISI	354	2.89 [2.07;4.41]	2.71 [1.73;4.43]	328	3.12 [2.19;4.32]	3.67 [2.55;5.33]*°	−0.11 [−0.33;0.36]	0.33 [−0.12;0.7] °
HOMA-IR	354	2.81 [1.94;4.28]	2.83 [1.79;4.45]	328	2.5 [1.84;4.1]	2.2 [1.41;3.32]*°	−0.04 [−0.4;0.44]	−0.25 [−0.75;0.14] °
Hep-IR*10^2	345	77.73 [51.88;117.07]	85.09 [53.68;133.32]	323	63.58 [47.28;98.98]	68.25 [48.15;100.34]*	−0.12 [−0.48;0.29]	−0.23 [−0.75;0.28]
iAUC I/G	351	25.37 [15.52;39.24]	22.24 [13.01;34.68]	323	20.33 [13.03;40.35]	18.32 [11.37;29.11]*	−0.17 [−0.59;0.5]	−0.21 [−0.67;0.32]
disposition index	350	52.04 [30.75;108.94]	46.77 [29.26;88.38]	323	51.78 [29.55;103.18]	51.06 [31.8;90.54]	−0.16 [−0.63;0.53]	0.13 [−0.45;0.74] °
Quantose IGT	328	61.48 [50.52;67.94]	63.21 [53.83;72.85]	327	57.79 [50.69;65.79]	61.19 [52.03;68.62]*	−0.06 [−0.19;0.1]	−0.08 [−0.25;0.08]
AUC-G (10^2*mmol min/l)	356	12.22 [10.88;14.5]	13.11 [11.49;15.28]	329	12.38 [11.01;14.3]	12.69 [11.22;14.4]*	0.04 [−0.1;0.14]	−0.06 [−0.24;0.07] °
AUC-I (10^3*pmol min/l)	364	84.22 [55.47;119.38]	83.57 [54.56;130.91]	328	75.88 [56.92;103.78]	60.47 [46.52;89.75]*°	0.01 [−0.21;0.28]	−0.28 [−0.69;0.05] °
Triglycerides (mmol/l)	368	1.32 [0.95;1.79]	1.23 [0.92;1.68]	325	1.31 [0.92;1.81]	1.09 [0.76;1.5]*°	−0.06 [−0.29;0.31]	−0.18 [−0.52;0.19] °
Cholesterol (mmol/l)	366	5.16 [4.43;5.86]	5.3 [4.55;5.99]	323	5.13 [4.34;5.92]	5.03 [4.31;5.79]*	−0.02 [−0.13;0.1]	−0.06 [−0.22;0.06]
HDL (mmol/l)	368	1.25 [1.05;1.46]	1.21 [1.05;1.44]	325	1.24 [1.01;1.54]	1.21 [1.02;1.44]	−0.01 [−0.15;0.09]	0 [−0.16;0.12]
LDL (mmol/l)	365	2.94 [2.5;3.98]	3.49 [2.79;4.17]	324	3.15 [2.48;3.98]	3.07 [2.61;3.85]*	0 [−0.19;0.17]	−0.09 [−0.29;0.1]

* Wilcoxon's test p-value PRE vs POST <0.05.
° Mann-Whitney's test p-value CT vs LI <0.05. All p-values were adjusted for FDR.

2.3. Metabolomics

In the DEXLIFE intervention study, a panel of 23 plasma metabolites was measured at baseline and at end of trial previously identified as markers glucose intolerance by Cobbs et al. [22] The complete list of metabolites is reported in Table S1 and includes the branched-chain amino acids and a number of their catabolites, plus glycine, tyrosine, 2-aminoadipic acid, α-hydroxybutyrate, and linoleoyl-glycerophosphocholine (LGPC or LysoPC(18:2)) and an unknown compound having a defined mass spectrometric signature, but no defined structure, X-12063, which was previously associated with insulin sensitivity [23] and IGT [22]. Absolute quantification of these metabolites was obtained by ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) analysis using internal standards labelled with stable isotopes as previously described [22]. The IGT metabolomic test called Quantose *IGT*™ was performed on all subjects pre and post intervention [22].

2.4. Machine learning models

Data from DEXLIFE were modeled by unsupervised and supervised machine learning techniques, namely k-means clustering and logistic regression, respectively.

K-means clustering was performed using three pre-intervention indices calculated on the 2 h-OGTT and related to increased risk of T2D [5,15,19], i.e., *i)* AUC-I as a marker of insulin secretion, and the two components of the oral disposition index, which are *ii)* ISI, and *iii)* iAUC I/G as a marker of pancreatic β-cell response to the glucose load. The clustering was done on all subjects that had complete dataset for the clustering variables, i.e., 350 subjects of the initial cohort of n = 373 (Table 1). Features were log-transformed and then centered to a mean of 0 and standard deviation of 1. We set 25 as the number of initial randoms starts. Clusters centroids coordinates are reported in Table S2. The consistency of our clustering was measured by using the silhouette analysis, a measure of how similar an object is to its own cluster compared to other clusters, and the Jaccard coefficients, that estimates cluster wise stability. Jaccard coefficients were calculated by resampling the data points using non-parametric bootstrap 100 times and then computing the bootstrap distribution of Jaccard similarities to the original clusters. Generally, stable clusters should yield a Jaccard similarity of >0.75 [24].

The EGIR-RISC cohort dataset was used to externally validate the clustering. In accordance with the method outlined Ahlqvist et al. [25], we focused on the subset that comprised of individuals (n = 1356) with complete data for the clustering variables. Within this subset, we allocated each individual to the DEXLIFE cluster that exhibited the smallest Euclidean distance from the centroids of the clusters. Further details are given in the Supplementary Materials.

A logistic regression model was trained to evaluate the ability of the pre-intervention indexes (predictors) used to perform the clustering i.e., ISI, AUC-I and iAUC I/G, in predicting a positive response to lifestyle intervention in DEXLIFE, defined as a binary outcome. Detailed methodology for model training and validation is provided in the Supplementary Materials. Briefly, response was defined *positive* if Delta (Post – Pre) of the area under the 2 h-OGTT curve of glucose (AUC-G) was lower than the quantile 0.33 of its distribution i.e., Delta AUC-G = −101.7 mmol min/L. Otherwise, it was defined *non-positive*. In other words, we considered the third of the subjects with the greatest decrease in Delta AUC-G to be positive responders. The predictive ability of the model was measured using accuracy and AUROC. Additional logistic regression models were trained and tested following the same methodology with the sole purpose of evaluating the additional predictive effect of omics variables, such as Quantose IGT or selected metabolites, with respect to the variables used to perform clustering.

2.5. Statistical analysis

Changes after the follow-up period were reported as Log2 fold changes (Log2 Post/Pre) or Delta (Post-Pre). Spearman rank correlation was used to establish association among different features. Pairwise differences between groups were analyzed by Mann-Whitney's test and pre vs post comparisons were conducted by paired Wilcoxon's test. Comparison among multiple groups were performed using Kruskal-Wallis' test. p-Values were adjusted for multiple comparisons i.e., by controlling for the false discovery rate (FDR), calculated according to the Benjamini–Hochberg procedure [26]. OGTT glucose and insulin levels in different clusters at baseline and deltas (post- pre) were compared using a two-way mixed ANOVA. Normality and homoscedasticity assumptions were checked using Shapiro-Wilk's and Levene's tests, respectively. Missing data were imputed using K-nearest neighbors' method (KNN). See Supplementary Materials for further details. All statistical and machine learning analyses were conducted using the R software (R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/), and data in tables were reported as medians and interquartile ranges (Table 2).

3. Results

3.1. Efficacy of lifestyle intervention in the DEXLIFE cohort

Of the 373 subjects initially enrolled in the DEXLIFE intervention study, 335 completed the follow-up (Fig. S1). Before the intervention, there were no significant differences between subjects allocated to CT and LI, after adjusting for multiple comparison (Table 1). Subjects enrolled in this study showed high heterogeneity in terms of insulin sensitivity and glucose tolerance: 114 subjects (30.6 %) had normal glucose levels, 149 (39.9 %) had isolated IFG, 61 (16.3 %) had IGT and 25 (6.7 %) were screen-detected with T2D during the first OGTT analysis. These individuals were included in the trial and assigned to lifestyle intervention (n = 22) or to the control group (n = 3). After 12 weeks of intervention, subjects assigned to lifestyle intervention showed improvement in several physiological features (Table 1), while no significant changes were observed in CT. LI significantly increased the VO_2max and reduced visceral fat, BMI, and circulating levels of LDL and triglyceride. Insulin and glucose concentrations during OGTT were significantly decreased in LI compared to CT. Consistently, insulin sensitivity was significantly improved in LI with respect to controls, as shown by the fold changes of both ISI and HOMA-IR. However, no significant changes were observed in iAUC I/G, or in the hepatic insulin resistance index (Hep IR). The disposition index showed a mild increase in LI (Table 1).

3.2. Cluster analysis identified four different groups in DEXLIFE cohort that responded differently to lifestyle intervention

The cluster analysis of the DEXLIFE cohort was based on pre-intervention indices of insulin sensitivity (ISI), glucose-stimulated insulin response (i.e. AUC-I during OGTT) and insulin secretion (iAUC I/G) and identified four clusters (CL1-CL4) with an average silhouette of 0.32 and a median Jaccard coefficient of 0.88, indicating that the clustering was reliable and stable (Fig. S2, Table S2). The four subgroups had similar age and sex distribution, and no difference in the distribution of individuals assuming antihypertensive or lipid lowering medication was observed (Table S3). Individuals in CL1 and CL2 were mainly characterized by non-obese subjects (average BMI < 30, Table 2). However, CL2 had higher glucose levels during OGTT compared to CL1, due to lower insulin concentrations, and showed the lowest iAUC I/G among the clusters (Fig. 1C-E, Table 2, S4, S5). Compared to CL1 and CL2, individuals in CL3 and CL4 not only had higher BMI, but also increased visceral fat accumulation, a worse aerobic fitness and worse metabolic

Table 2
Baseline anthropometric and clinical characteristics of subjects from DEXLIFE cohort who had complete datasets for the clustering variables.

	CL1	CL2	CL3	CL4	p-Value
N = 350	72	81	134	63	
SEX (M/F)	29/43	36/45	79/55	32/31	
Age	52[47.5;57.25]	57[49;65]	54[48.25;64.75]	52[48;62.5]	0.0332
Sys BP (mm/Hg)	128[120;140]	130[120;140]	136[128;145]	136[128;146]	0.0002
Dia BP (mm/Hg)	81.45[80;88]	80[76.11;90]	84[80;90]	86[80;90]	0.1374
BMI (kg/m^2)	28.65[26.73;31.04]	28.09[25.07;30.82]	31.82[29.58;34.9]	34.28[30.91;37.79]	<0.0001
Weight (kg)	84.05[74.6;96.4]	79.5[70.1;88.7]	93.95[85.12;105.38]	98.1[88.35;112.4]	<0.0001
VO_2Max (ml/kg min)	29.02[25.33;36.2]	29.1[24.1;35]	27.5[22.35;32.81]	27.03[22.6;30.98]	0.0131
Fat (kg)	38.34[32.85;44.25]	35.14[27.57;42.87]	37.8[32.45;45.41]	40.35[35.74;46.78]	0.0006
Subcutaneous Fat (cm)	2.74[2.3;3.54]	2.44[1.92;3.13]	2.84[2.24;3.53]	3.29[2.52;3.68]	0.0011
Visceral Fat (cm)	6.28[5.2;7.27]	6.36[5.24;7.45]	7.81[6.81;9.37]	8.1[7.14;9.05]	<0.0001
Glucose 0′ (mmol/l)	5.52[5.1;5.85]	5.53[5.16;5.89]	5.88[5.47;6.39]	6[5.71;6.44]	<0.0001
Insulin 0′ (pmol/l)	1630.62[984.16;2736.06]	550.06[354.68;957.6]	348.39[222.05;470.22]	389.66[286.34;629.13]	<0.0001
ISI	1.37[1.14;1.55]	1.34[1.15;1.65]	1.12[0.99;1.34]	1.15[1;1.32]	<0.0001
HOMA-IR	3.34[2.73;4.01]	3.18[2.52;3.67]	3.54[2.75;4.2]	3.46[2.86;4.26]	0.0768
Hep-IR*10^2	6482.22[4469.35;10,194.19]	4437.07[2911.06;5761.9]	9913.69[7327.51;12,133.03]	20,256.96[13,299.54;24,372]	0.0332
iAUC I/G	456.44[295.62;722.96]	123.25[82.68;175.75]	178.3[129.13;240.88]	367.85[288.29;542.83]	0.0002
disposition index	1630.62[984.16;2736.06]	550.06[354.68;957.6]	348.39[222.05;470.22]	389.66[286.34;629.13]	0.1374
Quantose IGT	57.09[45.74;62.6]	57.38[50.62;66.3]	64.64[57.8;73.61]	66.54[57.06;77.11]	<0.0001
AUC-G (10^2*mmol min/l)	1064.55[998.78;1113.04]	1220.55[1095;1382.7]	1418.55[1301.7;1598.03]	1418.7[1230.3;1569.23]	<0.0001
AUC-I (10^3*pmol min/l)	63,828.6 [53,073.56;82,111.39]	46,178.7 [33,987.6;52,963.35]	99,155.33 [80,987.33;122,550.94]	183,965.4 [148,020.75;241,040.25]	0.0131
Triglycerides (mmol/l)	1.1[0.86;1.64]	0.99[0.77;1.31]	1.44[1.08;1.95]	1.57[1.22;2.08]	0.0006
Cholesterol (mmol/l)	5.36[4.72;6.05]	5.24[4.56;5.8]	5.29[4.44;5.93]	5.23[4.41;6.1]	0.0011
HDL (mmol/l)	1.37[1.14;1.55]	1.34[1.15;1.65]	1.12[0.99;1.34]	1.15[1;1.32]	<0.0001
LDL (mmol/l)	3.34[2.73;4.01]	3.18[2.52;3.67]	3.54[2.75;4.2]	3.46[2.86;4.27]	<0.0001

Kruskal-Wallis' test p-values, after FDR adjustment are reported.

profile, characterized by low insulin sensitivity but high AUC-I to compensate for the increased insulin resistance (Fig. 1A-B, Table 2, S4). Furthermore, these clusters included the higher proportion of subjects with abnormal glucose concentrations (Fig. 1F). CL3 and CL4 had higher triglycerides, and lower HDL cholesterol. The disposition index and 1-h glucose, an important marker of T2D risk [27], showed a stepwise trend from CL1 to CL4, with CL4 exhibiting the least favorable pre-intervention metabolic profile (Table 2, S4, S5, Fig. 1).

The response to 12-week lifestyle intervention was highly heterogeneous within the four clusters (Table 3, S6). In contrast, no differences among the clusters were observed within the control group (Table S7). Clusters in the LI subgroup showed no significant differences in the adherence to the intervention, with similar total time of physical exercise (Table S8). Moreover, they presented a significant but similar improvement in VO_2max, reduction in body weight and total fat, although the decrease in visceral fat was greater in LI-CL4. Despite these similarities, the responses to lifestyle intervention in terms of glucose tolerance were different in the four clusters (Table 3, S6, S9). In LI-CL3 and LI-CL4, glucose and insulin concentrations were significantly decreased during OGTT at each time point (Fig. 1G-H) compared to controls, even after adjusting for body weight loss (Table S10). Consistently, LI-CL3 and LI-CL4 achieved similar improvements in all indices of insulin sensitivity (i.e., HOMA-IR, Hep-IR, ISI), iAUC-I/G and AUC-I, while disposition index was significantly improved only in LI-CL4 (Fig. 1J-L). In contrast, LI-CL1 had similar glucose levels during OGTT, but a decrease in iAUC I/G due to lower insulin concentrations vs pre-intervention values at time 60′ and 120′ and AUC-I. LI-CL2 i.e., the cluster with the lowest insulin response at pre-intervention and high insulin sensitivity, showed no significant changes in either glucose or insulin curves or insulin sensitivity indices compared with pre-intervention, nor differences compared with controls (Table 3). Regarding glucose tolerance, a higher percentage of individuals in LI-CL3 and LI-CL4 obtained an improvement after 12 weeks (34 % and 39 %, respectively, Fig. S3) compared to LI-CL2 or controls (improvement in 18 % and 21 % respectively) or LI-CL1 where most of subjects remained almost stable (improvement in 12 %). On the other hand, CT had a high percentage of individuals that worsened their glucose tolerance (15 %), and this was relevant in CT-CL3 and CT-CL4, i.e., the clusters that benefit most of lifestyle intervention. It is of note that also LI-CL2 had a high percentage of individuals that worsened their glucose tolerance (15 %, similar to CT), much higher than in the other clusters (LI-CL1: 0.02 %, LI-CL3: 10 %, LI-CL4:7 %).

Circulating triglyceride concentrations were significantly reduced in LI-CL1, LI-CL2 and LI-CL3 after lifestyle intervention, while total cholesterol concentration was reduced only in LI-CL2 and LI-CL3 with respect to pre-intervention and control values (Table 3).

3.3. The four clusters were defined by distinct metabolomics signatures

Changes in metabolite concentration and "Quantose IGT", a metabolomic index of impaired glucose tolerance, might be used as markers of improvement/worsening of metabolic status. In the DEXLIFE study, a set of 23 metabolites associated with IGT and T2D were measured pre and post lifestyle intervention and in the control group. Pre-intervention metabolite concentrations and "Quantose IGT" were significantly different in the four clusters (Kruskal-Wallis test p-value <0.0001, Fig. 2A), consistently with differences observed in terms of glucose tolerance among the clusters. After 12 weeks, independent of the clusters, there were no significant changes in the control arm (Table S7). Focusing on the lifestyle arm, significant changes in the concentration of several metabolites were mainly observed in CL3 and CL4 (Fig. 2B) and such changes correlated with improvements in insulin sensitivity, secretion, and glucose control (Fig. 2C). In particular, the increase of 3-hydroxybutyrate observed in CL3 and CL4 was associated with the increase in ISI and VO_2max, and the reduction in body weight. The increase of glycine and the reduction of α-ketoglutarate were significantly correlated with the reduction of both AUC-G and AUC-I, and the improvement of disposition index, while the reduction of "Quantose IGT" significantly correlated with AUC-G (rho = 0.24, p-value = 0.0002, Fig. 2C). Most of these associations were still significant after adjusting for weight loss (Fig. S3). No change in metabolite concentrations was observed in the CT group (Table S7).

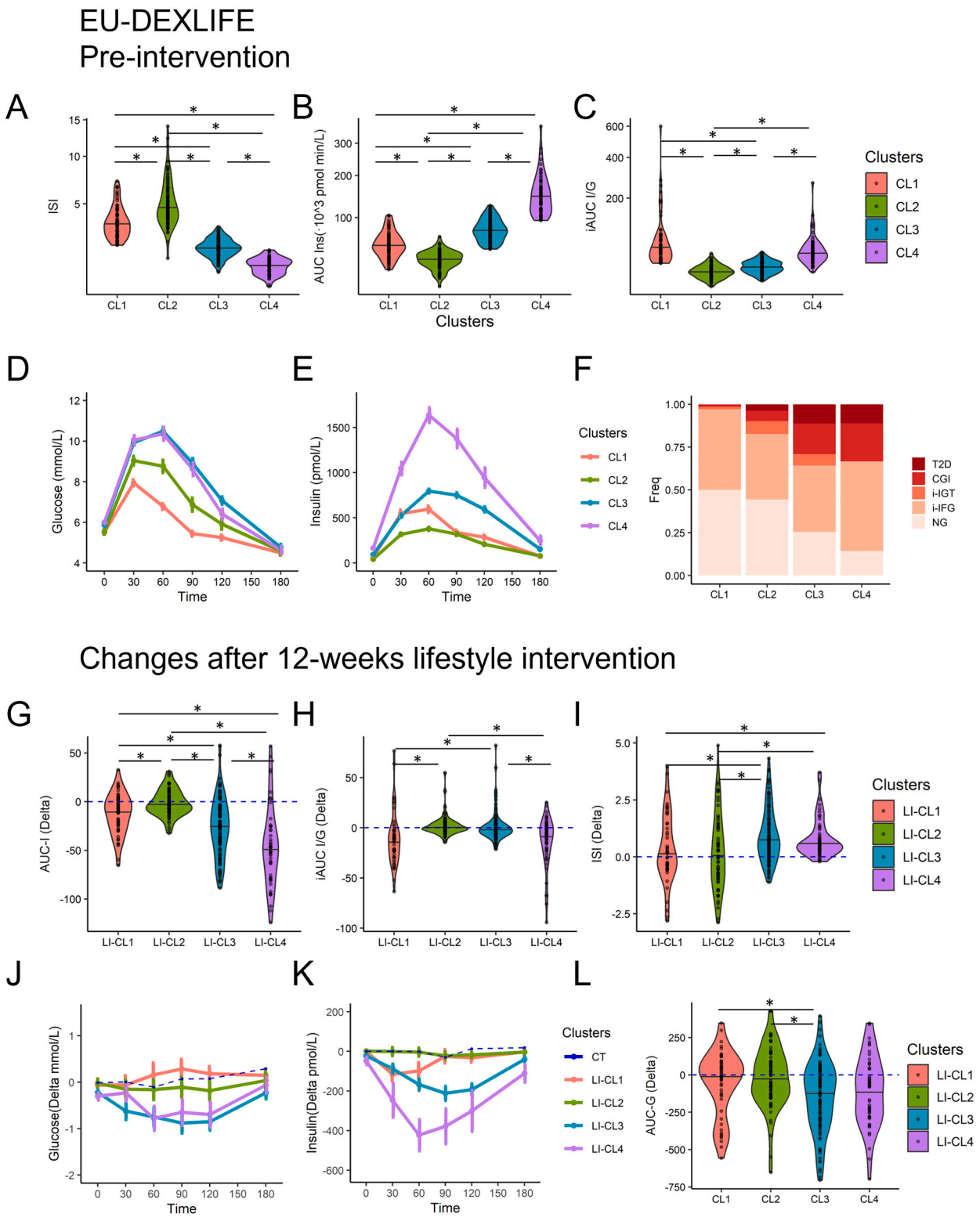

Fig. 1. Cluster Analysis in the DEXLIFE cohort at baseline (LI and CT) and after 12 weeks (LI). In panel A, B and C, boxplots for the four clusters of the clustering variables ISI, AUC-I, and iAUC I/G at baseline, respectively. * Mann-Whitney's test p-value <0.05, after FDR adjustment. In panel D and E, glucose and insulin curves during OGTT at baseline in the four clusters were reported as mean ± standard error (statistically significant differences are reported in Table S5). In panel F, the distribution of glucose control at baseline in the 4 clusters. Individuals were classified as normal glucose tolerance (NG), i-IFG, isolated IGT (i-IGT), combined glucose impairment (CGI, defined as IFG and IGT) or T2D. A small number of subjects (8.2 %) with T2D were screen-detected during the first OGTT analysis. Panel G-L: Changes after 12 weeks in the subgroup assigned to lifestyle intervention in the DEXLIFE cohort. Panel G, H and I show deltas (Post – Pre) of the clustering variables ISI, AUC-I and iAUC I/G in the four clusters. Dotted blue lines indicate Delta equal to 0. * Mann-Whitney's test p-value <0.05, after FDR adjustment. In panel J and K, deltas of glucose and insulin concentration levels during OGTT were reported as mean ± standard error. Dotted blue lines represent the OGTT curves in the control subgroup. Statistically significant differences are reported in Table S6, S9. Panel L shows the Delta (Post – Pre) in AUC-G. * Mann-Whitney's test p-value <0.05, after FDR adjustment. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
Delta (POST-PRE) of anthropometric and clinical characteristics of DEXLIFE study participants assigned to the lifestyle intervention, having complete data for clustering.

	LI-CL1		LI-CL2		LI-CL3		LI-CL4		p-Value
Sys BP (mm/Hg)	−1.78[−18;4.15]		−2.13[−12;9.5]		−6[−20;4]	*	0[−12.19;10]		0.1758
Dia BP (mm/Hg)	0[−10;2]		−2[−9.1;1.76]		−6[−10;2]	*	−2[−10;5]		0.4197
BMI (kg/m^2)	−1.04[−1.61;-0.33]	*§	−0.72[−1.46;0]	*§	−1.69[−2.38;-0.82]	*§	−1.38[−2.26;-0.45]	*§	0.0002
Weight (kg)	−3.2[−4.9;-0.8]	*§	−1.8[−3.8;0]	*§	−4.4[−7;-2.2]	*§	−3.85[−6.55;-0.87]	*§	0.0003
VO_2Max (ml/kg min)	3.4[−0.6;6.3]	*§	1.52[−0.93;6.02]	*	3.03[0.03;5.83]	§	2.76[−0.02;6.85]	*§	0.6196
Fat (kg)	−1.16[−3.17;-0.07]	*§	−1.48[−3.38;-0.27]	*§	−2.25[−3.72;-1.13]	*§	−1.99[−5.72;-0.47]	*§	0.2357
Subcutaneous Fat (cm)	−0.22[−0.64;0.18]		−0.34[−0.71;0.12]	*	−0.33[−0.57;-0.04]	*	−0.44[−1.03;-0.12]	*	0.163
Visceral Fat (cm)	−0.48[−1.73;0.23]	*	−1.02[−1.91;-0.13]	*	−1.68[−2.47;-0.3]	*§	−2.01[−2.95;-0.46]	*§	0.0046
Glucose 0′ (mmol/l)	−0.06[−0.43;0.13]	*	−0.02[−0.25;0.4]		−0.22[−0.59;0.08]	*§	−0.3[−0.71;-0.01]	*§	0.0004
Glucose 30′ (mmol/l)	−0.06[−0.81;0.83]		−0.15[−0.87;0.95]		−0.62[−1.62;0.72]	*§	−0.23[−0.82;0.5]		0.163
Glucose 60′ (mmol/l)	0.16[−1;1.21]		−0.16[−1.38;0.6]		−0.75[−2.19;0.35]	*§	−0.78[−2.16;0.2]	*§	0.0049
Glucose 90′ (mmol/l)	0.29[−0.48;1.14]		−0.11[−1.11;1.12]		−0.88[−2.27;0.22]	*§	−0.65[−2.05;0.81]	*§	0.0004
Glucose 120′ (mmol/l)	0.18[−0.68;0.74]		−0.18[−1.27;0.9]		−0.85[−1.89;0.48]	*§	−0.7[−1.71;0.21]	*§	0.0016
Glucose 180′ (mmol/l)	0.15[−0.41;0.56]		0.04[−0.72;0.75]		−0.23[−0.99;0.38]	*	−0.06[−0.95;0.55]		0.1314
Insulin 0′ (pmol/l)	0.63[−14.97;11.01]		0.77[−6.4;11.34]		−22.55[−43.59;2.02]	*§	−47.04[−73.5;-23.98]	*§	<0.0001
Insulin 30′ (pmol/l)	−115.5[−228.5;45.9]	*	−0.75[−71.5;71.6]		−87.8[−241.4;30.3]	*	−246.29[−477.65;-3.92]	*§	<0.0001
Insulin 60′ (pmol/l)	−98.08[−298.16;44.1]	*	−3.8[−91.93;67.4]		−167[−379.9;13.42]	*§	−422.9[−873.15;-198]	*§	<0.0001
Insulin 90′ (pmol/l)	−24.8[−177.5;62.1]		−21.95[−116.06;52.55]		−211.8[−472.5;-34.8]	*§	−378.25[−629.11;14]	*§	<0.0001
Insulin 120′ (pmol/l)	−32.11[−104.94;14.1]	*	−17.18[−112.08;45.92]		−193.3[−394;-31.8]	*§	−300.88[−654.7;3.47]	*§	<0.0001
Insulin 180′ (pmol/l)	−2.39[−25.76;22.59]		−3.76[−44.54;13.78]		−39.62[−86.36;-1.1]	*§	−110.72[−296.48;-19.37]	*§	<0.0001
ISI	0.15[−0.41;1.47]		−0.23[−1.37;1.23]		0.73[0.09;1.64]	*§	0.56[0.31;1.06]	*§	0.0001
HOMA-IR	0.01[−0.56;0.37]		0.08[−0.25;0.5]		−0.88[−1.88;0.09]	*§	−1.8[−2.82;-0.9]	*§	<0.0001
Hep-IR*10^2	−1552.37 [−2809.26;792.4]	*	85.15 [−1513.22;1690.73]		−2724.03 [−6252.03;986.03]	*§	−5953[−10,934.75;455.69]	*§	<0.0001
iAUC I/G	−152.78[−295.38;-69.56]	*§	−4.29[−33.46;39.04]		−19.04[−62.02;35.82]		−77.18[−227.87;15.84]	*§	<0.0001
disposition index	−422.27[−927.16;-62.53]	*§	−43.83 [−238.52;205.44]		47.47[−28.07;206.83]	*§	111.12[−10.26;330.48]	*§	<0.0001
Quantose IGT	−3.45[−7.77;0]	*	−0.08[−7.29;6.09]		−3.55[−10.36;2.63]	*	−1.08[−11.39;1.49]	*	0.1861
AUC-G (10^2*mmol min/l)	−5.7[−140.4;85.95]		−44.55[−149.36;90.3]		−128.1[−301.5;25.05]	*§	−76.08[−278.63;51.86]	*§	0.0055
AUC-I (10^3*pmol min/l)	−8967 [−24,443.25;2143.65]	*	−3378.83 [−10,231.76;6891]		−25,678.5[−44,464.5;-6867]	*§	−51,830.7[−86,511.49;-29,397.15]	*§	<0.0001
Triglycerides (mmol/l)	−0.12[−0.32;0.11]		−0.06[−0.34;0.14]		−0.16[−0.46;0.23]	*	−0.17[−0.45;0.11]	*	0.6657
Cholesterol (mmol/l)	−0.35[−0.64;0.14]	*	−0.24[−0.7;0.14]		−0.23[−0.87;0.2]	*	−0.25[−0.67;0.4]		0.9154
HDL (mmol/l)	0.03[−0.12;0.12]		−0.02[−0.17;0.14]		0.03[−0.12;0.2]		0.02[−0.11;0.1]		0.8616
LDL (mmol/l)	−0.19[−0.47;0.26]		−0.26[−0.63;0.22]		−0.2[−0.67;0.2]	*§	−0.24[−0.78;0.13]	*§	0.8755

Kruskal-Wallis' test p-values, after FDR adjustment, are reported. * Significant changes vs baseline values, Wilcoxon test (paired) < 0.05, after FDR correction. § Significant changes vs control group, Mann-Whitney's test <0.05, after FDR correction.

3.4. High insulin resistance and concentrations were predictive of beneficial effects of lifestyle intervention in glucose control

The pre-intervention parameters used to perform unsupervised clustering, i.e., ISI, iAUC I/G and AUC-I, were significantly correlated with the improvements in glucose tolerance, insulin sensitivity and secretion in the LI group (Fig. 3A). Logistic regression model A, used to evaluate the predictive power of pre-intervention features on response to lifestyle intervention, was able to discriminate positive vs non-positive response to lifestyle intervention with good performance and accuracy during repeated cross-validation (average AUROC = 0.72) and testing (AUROC = 0.79, 95 % CI: [0.65,0.93]; accuracy 70 %), as reported in Fig. 3B-C and Table S11. The observed wide AUROC CIs are due to the small size of the test set. Consistently, we noticed that the percentage of subjects with a positive response was not equally distributed among the clusters. The percentage of positive responses in LI-CL3 and LI-CL4 was almost twice as high as in LI-CL1 and LI-CL2 (41 % and 48 % vs 12 % and 25 %, respectively, Fig. S5). Additional classification models were trained and tested to account for multicollinearity among the predictors in Model A (Model B, Table S12) and to evaluate the additional predictive effect of omics variables, like Quantose IGT and the metabolites that were significantly associated (Fig. S4) with reduced AUC-G (Model C-E, Table S11). We found that the performance of these models, in terms of accuracy and AUROC, was similar to or worse than Model A, as reported in Table S11 (Models B-E), suggesting that the inclusion of metabolites and/or "Quantose IGT" did not provide the additional information needed to explain the variability in response to intervention or enhance Model A, which relied solely on OGTT-based indices.

3.5. External validation in the EGIR-RISC cohort

The EGIR-RISC cohort (n = 1538) was used to support and validate these findings. For cluster analysis to be clinically useful, subjects should be assigned to the clusters without de novo clustering of the whole cohort. Thus, individuals from the EGIR-RISC cohort that had complete data for the clustering variables were assigned to the "closest" DEXLIFE cluster as described in the methods (Fig. S6, S7). Since the EGIR-RISC cohort had in general more favorable glucose control than the DEXLIFE, most of the subjects were allocated to CL1 and CL2. CL3 and CL4

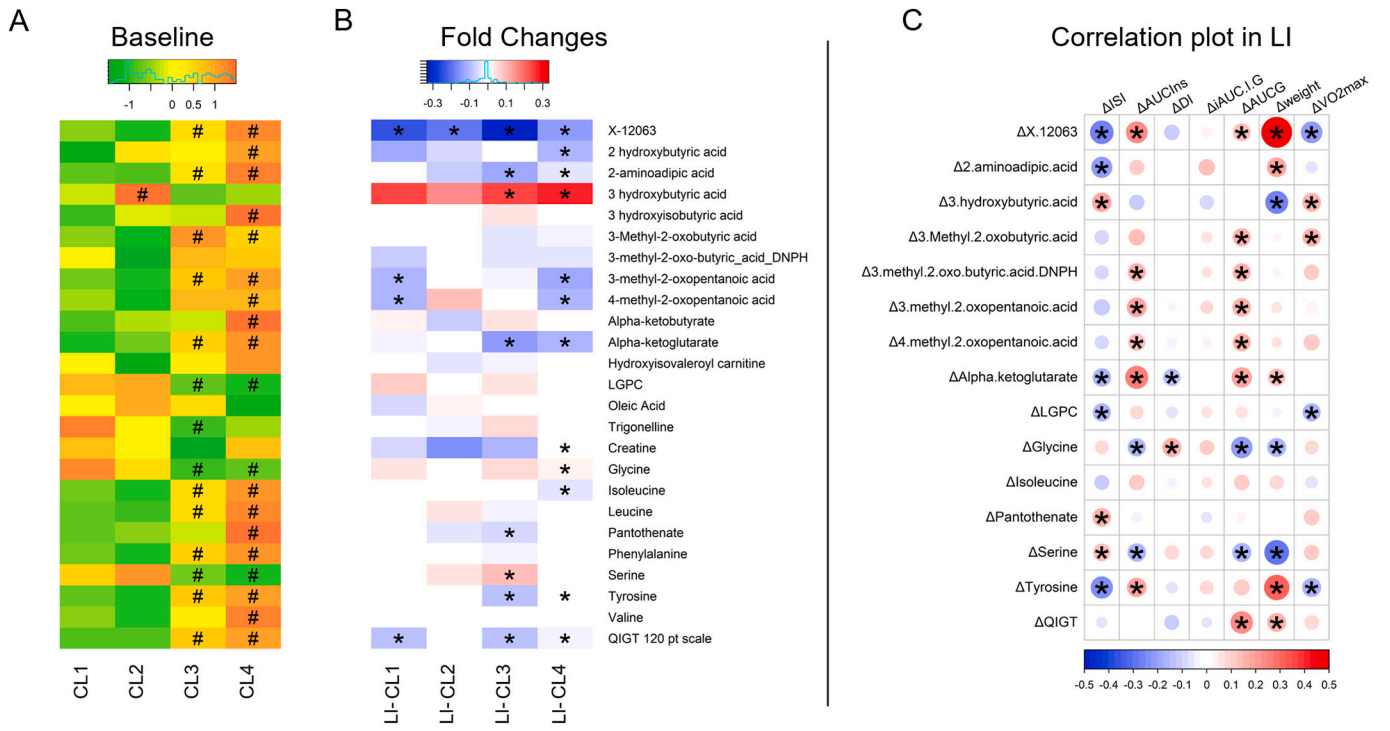

Fig. 2. Metabolomic signature of the clusters at baseline in the DEXLIFE cohort (LI and CT). In panel A, distribution of metabolites and Quantose IGT (QIGT) in the four cluster at baseline; # Mann-Whitney's test p-value vs CL1 < 0.05. In panel B, fold changes (log2 Post/Pre) of metabolites and Quantose IGT (QIGT) in lifestyle intervention arm; * Paired Mann-Whitney's test p-value pre vs post <0.05. In panel C, Spearman correlation plot between Deltas (pre – post) of metabolites significantly changed in CL3 or CL4 after the lifestyle intervention and Deltas of metabolic indices of insulin sensitivity secretion and glucose control, body weight and VO_2max in the LI subgroup; * Spearman correlation test p-value <0.05. p-Values in Panel A and B were adjusted for FDR.

were grouped together (CL3/CL4), because only 7 subjects were assigned to CL4. Despite the differences in distribution, the allocated clusters in the EGIR-RISC cohort showed a similar metabolic behavior to the one observed in the DEXLIFE study (Fig. 4A-F, Table S13). At follow-up after 3 years, 371 individuals displayed weight loss and we focused on this subgroup for the comparison with the LI group of DEXLIFE cohort. Consistent with DEXLIFE findings, we observed that CL3/CL4 achieved the best improvement in insulin sensitivity (pre vs post in CL3/CL4, $p < 0.001$) and disposition index, despite values not reaching statistical significance (Table S14). The OGTT curves of glucose and insulin changed similarly to those in the DEXLIFE study (Fig. 4A-F). In addition, we found that the association observed between baseline values and changes in glucose tolerance, insulin sensitivity, and insulin secretion aligned with the results observed in the DEXLIFE study (Fig. S8).

4. Discussion

The inter-individual variability in the response to lifestyle intervention constitutes an obstacle and source of delay for an efficient prevention and treatment of T2D. In this work, we analyzed the pre-intervention sub-phenotypes of subjects at high risk of T2D by machine learning and clustering analysis based on pre-intervention characteristics like insulin sensitivity and insulin secretion indexes (i.e., ISI, AUC-I and iAUC I/G). We identified four reproducible clusters and showed that they responded differently to lifestyle intervention and consequently had different odds of success in improving glucose metabolism (Fig. S3, S5). This approach is novel compared to previous studies that usually use a retrospective approach and analyze the baseline characteristics of individuals that responded to the intervention. Moreover, while previous studies focused mainly on individuals with IGT as group of subjects at high risk of T2D, the DEXLIFE cohort included, besides IGT, individuals with a large spectrum of glucose concentrations, but at high risk of T2D based on FINRISK score. We showed that the baseline sub-phenotype is important for the response to personalized dietary intervention and exercise in terms of improvement in glucose metabolism.

We demonstrated that high risk subjects belonging to LI-CL3 and LI-CL4 (i.e., who had low pre-intervention insulin sensitivity, but high glucose-stimulated insulin response and also higher BMI), showed the best response to lifestyle intervention, improving both glycemic control (measured by AUC-G) and iAUC-I/G in relation to insulin sensitivity (disposition index), but otherwise worsen glycemic control in the control group. The results of the clustering and the impact of weight loss were validated in a second cohort, i.e., the observational study EGIR-RISC, which included individuals without diabetes or hypertension at baseline followed for 3 years. Among individuals who had a spontaneous weight reduction at 3 years, those with a high baseline insulin response to OGTT and insulin resistance showed the greatest improvement in glucose tolerance confirming the results of the DEXLIFE.

It is worth also noticing that LI-CL3 and LI-CL4 had the highest pre-intervention 1 h-glucose, a further marker of increased risk of T2D [27], and the best improvement not only in 1 h-glucose but also reduction in insulin resistance and visceral fat, in agreement with the findings of Sandforth et al. [28] (Table 3, S6). Subjects in LI-CL1, with high insulin sensitivity and preserved secretion, showed no changes in insulin sensitivity or glucose profile during OGTT, but reduced disposition index due to reduced AUC-I, although both pre- and post-intervention metabolic control was better than LI-CL3 and LI-CL4. LI-CL2 also showed in average minimal changes in glucose AUC, probably due to a good metabolic control both pre- and post-intervention. However, a high percentage of individuals in LI-CL2 worsened their glucose tolerance (15 %, similar to CT), much higher than in the other clusters, probably because of impaired insulin secretion, despite high pre-intervention insulin sensitivity.

The reproducibility of these results was tested and validated in the EGIR-RISC cohort where subjects were assigned to the "closest"

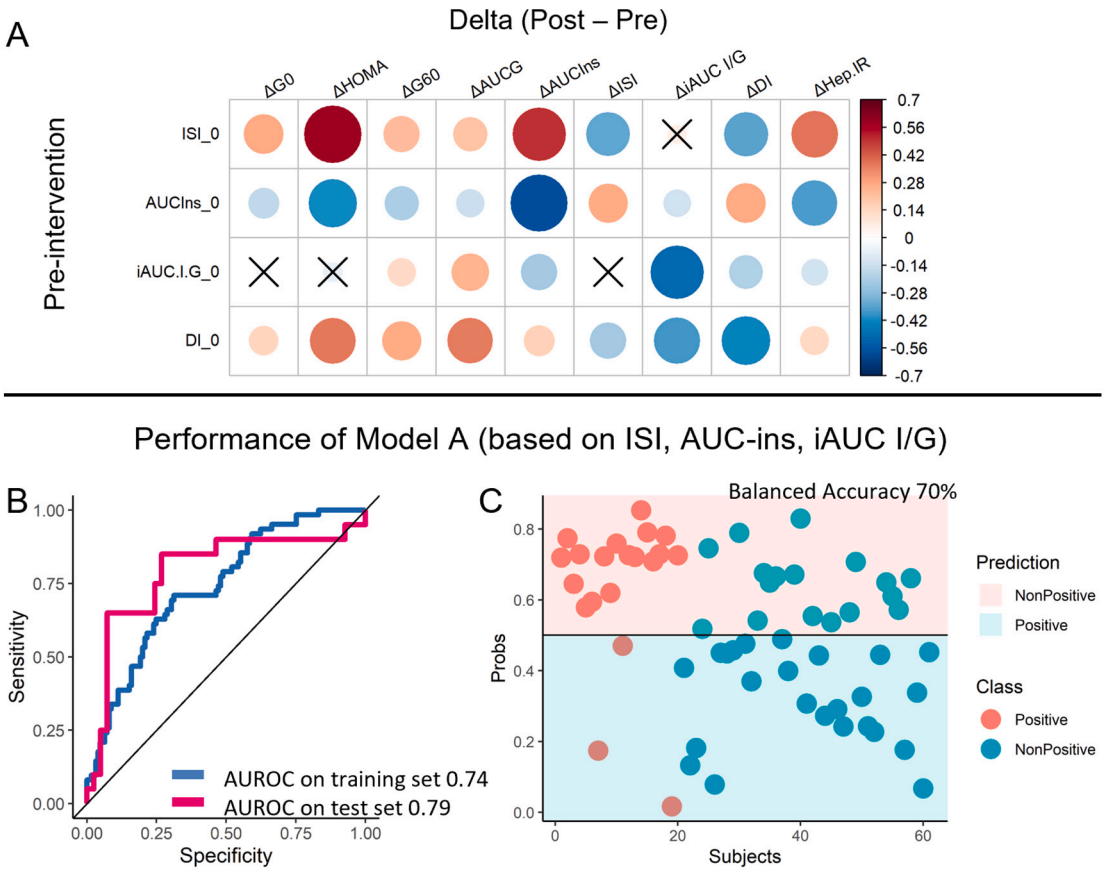

Fig. 3. Pre-intervention insulin metabolism was predictive of the beneficial effects of lifestyle intervention in the DEXLIFE cohorts. A) Correlation matrix of the baseline features used for clustering and as predictors in Model A, and the baseline disposition index (DI_0), with changes (Delta Post -Pre) in glucose metabolism in the LI subgroup. B) ROC curves of Model A to predict a positive response to the lifestyle intervention, based on delta AUC-G, applied to the whole training set (in blue) and the test set (in red). C) Accuracy of the prediction of Model A on the test set. For each subject in the test set (x-axis), the prediction of his response (y-axis) is reported. Scores' colour (*Class*) indicates the *positive* (red) or *non-positive* (blue) response to the intervention, while the colour of the area where each score is placed (*Prediction*) represents the model's prediction (*positive* in pink, or a *non-positive* in light blue): a red (blue) point placed in the pink (light blue) area represents a correct classification, a red (blue) point placed in the light blue (pink) area represents a misclassification. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

DEXLIFE cluster without de novo clustering of the whole cohort using the approach previously outlined by Ahlqvist et al. [25] Moreover, the correlation analysis in both DEXLIFE and EGIR-RISC cohorts showed that the decrease in AUC-G was associated with low pre-intervention levels of ISI, but high levels of AUC-I and iAUC I/G (Fig. 3A, S8). These findings were also strengthened by the supervised modeling (Fig. 3B-C), suggesting that these associations had also a predictive power. However, given the small sample size, especially the test set, or the lack of an external validation cohort, further studies will be required to validate the logistic regression models in larger cohorts.

All together, these results indicate that pre-intervention phenotype is crucial for reducing the risk of developing T2D associated to glycemic control and that weight loss alone is not sufficient to obtain an improvement in glucose tolerance in absence of preserved capacity of β-cell insulin secretion in response to glucose changes. This result is in line with previous findings showing the association between pre-training markers of insulin secretory function and training-induced improvements in glycemic control and β-cell function in subjects with prediabetes and T2D [13,29] and explained why subjects in LI-CL2, who had low pre-intervention AUC-I and iAUC I/G, did not show a significant change in AUC-glucose after lifestyle intervention, despite weight loss and high insulin sensitivity. In the DIRECT Study, the remission of T2D after weight loss was also associated with the capacity of the β-cell to recover the glucose-stimulated insulin response [30]. Stefan et al [15] identified a high-risk phenotype, consisting of low disposition index or insulin resistant steatotic liver disease, in which the decrease in AUC-G after lifestyle intervention was milder than in other subjects, but Fritsche et al. recently showed that an intensive lifestyle intervention could compensate this mild response and induce a superior improvement of glucose metabolism after one year [31].

Although possible sources of heterogeneity in the response of blood glucose control to lifestyle intervention could also reside in type, intensity, and frequency of physical exercise and/or weight loss [13,32], this was not the case in DEXLIFE since the total time of exercise and adherence to the program was similar in the four clusters (Table S8), as well as the reduction of body fat (Table 3), nor in the EGIR-RISC that is an observational study (Fig. 4, Table S14). Several cardiometabolic risk factors improved independently of weight loss after lifestyle intervention, especially in LI-CL3/4, as shown by the results of the sensitivity analysis reported in Table S10 and in agreement with Solomon et al. [13] The reduction of visceral fat was higher in responder clusters (LI-CL3 and LI-CL4) in DEXLIFE, although not significant. Thus, the improvements might be mediated by a reduction in hepatic or pancreatic fat, as shown the DIRECT study [30], but unfortunately these fat depots were not measured in these studies.

In this work, we also analyzed the effect of lifestyle intervention and weight loss on the serum concentrations of a panel of 23 metabolites (Table S1) previously shown to be associated with prevalent or incident type 2 diabetes or dysglycemia in in-house nonbiased global metabolomic profiling studies (including EGIR-RISC, Botnia [33], METSIM

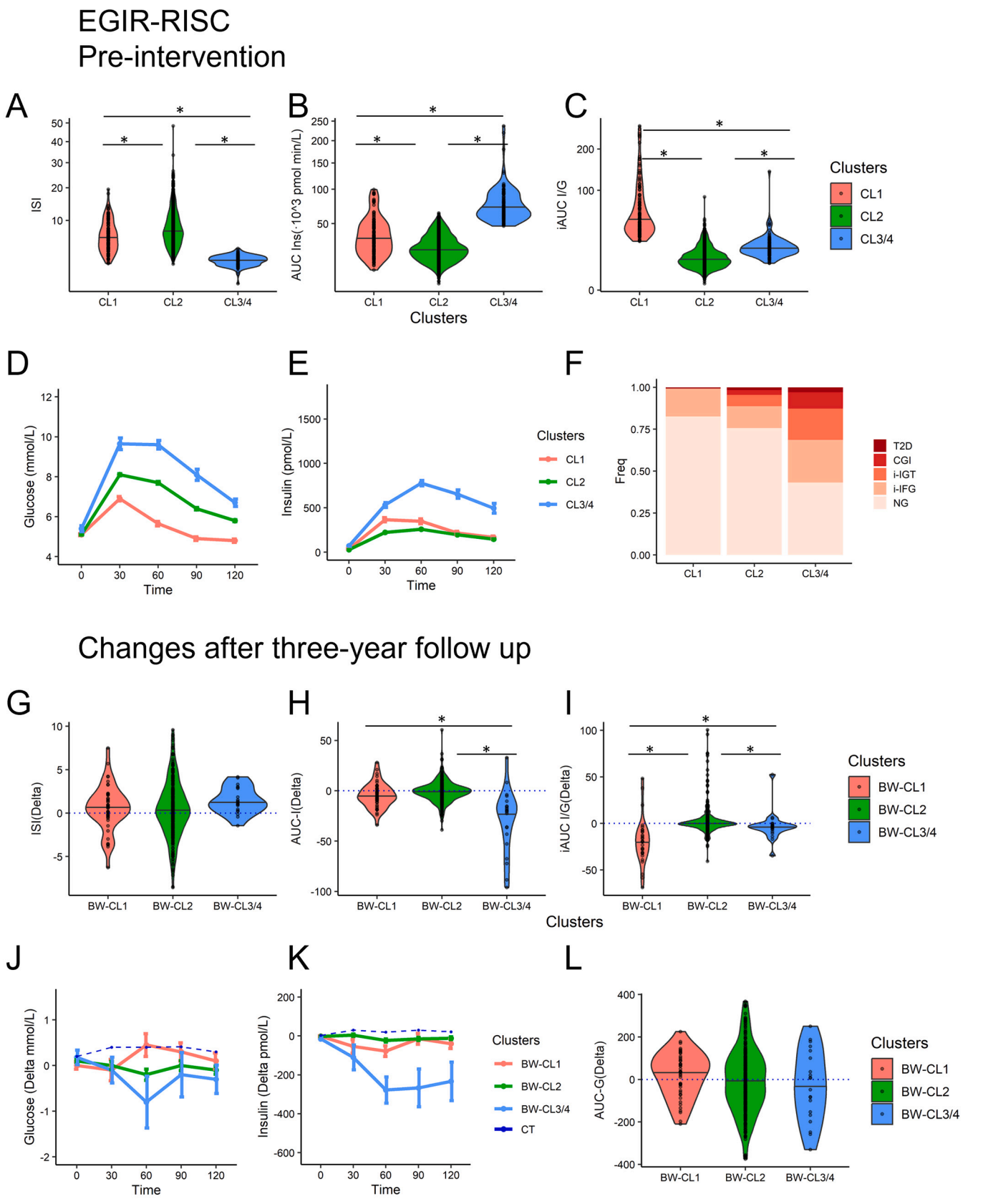

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Fig. 4. Results of clusters' assignment in the EGIR-RISC cohort at baseline and after 3 years in the subgroup of individuals who experienced weight loss. In panel A, B and C, boxplots the clustering variables ISI, AUC-I, and iAUC I/G at baseline, respectively. * Mann-Whitney's test p-value <0.05, after FDR adjustment. In panel D and E, glucose and insulin curves during OGTT at baseline in the clusters were reported as mean ± standard error. Statistically significant differences are reported in Table S13. In panel F, the distribution of glucose control at baseline in the 4 clusters. Individuals were classified as normal glucose tolerance (NG), i-IFG, isolated IGT (i-IGT), combined glucose impairment (CGI, defined as IFG and IGT) or T2D. Panel G-L: changes after 3 years in individuals the EGIR-RISC cohort that had a spontaneous body-weight loss. Panel G, H and I show log2 fold changes of the clustering variables ISI, AUC-I and iAUC I/G in the clusters. Dotted blue lines indicate log2 fold change equal to 0. * Mann-Whitney's test p-value <0.05, after FDR adjustment. In panel J and K deltas (Post – Pre) of glucose and insulin concentration levels during OGTT were reported as mean ± standard error. Dotted blue lines represent the OGTT curves in the control subgroup i.e., individuals that increased or maintained their body weight after 3 years. Statistically significant differences are reported in Table S14. Panel L shows the Delta (Post – Pre) in AUC-G. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

[34] and DMVhi) and selected for the development of a metabolite-based test for IGT by Cobb et al. [22] We investigated whether such biomarkers exhibit changes in the opposite direction with the risk of T2D. Pre-intervention clusters with a worse metabolic profile i.e., CL3 and CL4 presented increased BCAA and decreased glycine and serine concentrations, in agreement with previous findings [35,36]. α-keto-glutarate, an intermediate of Krebs cycle usually increased in subjects with metabolic dysfunction [35] and insulin resistance [23], was also increased in CL3 and CL4. After lifestyle intervention we observed that this set of metabolites changed along with the improvement in glucose metabolism, with an increase in the serum concentration of 3-hydroxy-butyrate, a ketone body produced by the hepatic oxidation of fatty acids. Consistently, the major changes were observed in LI-CL3 and LI-CL4, while no change was shown in the CT group.

We also evaluated levels of serine and glycine that are negatively associated with insulin resistance and hepatic dysfunction [35,37]. Moreover, the supplementation of glycine together with acetyl cysteine increased glutathione and improved markers of oxidative stress, mitochondrial function, inflammation, and physical function in older adults [38] while supplementation of serine, a precursor of glycine, improved hepatic function and decreased hepatic fat [39]. In this study, LI increased serum glycine and the change was associated with the improvement in glucose metabolism (Fig. 2), even after adjustment for body weight loss (Fig. S4).

The analyses of this study rely on common OGTT-based indices of insulin sensitivity and secretion that can be easily applied. However, ISI, AUC-I and iAUC I/G need to be calculated from frequently sampled OGTT. Metabolomic signature was proposed as alternative/complementary to metabolic tests like OGTT or euglycemic hyperinsulinemic clamp since it can be performed in one single sample obtained in fasting state [22]. Thus, we evaluated the predictive values of target metabolomics on a single fasting sample. The Quantose IGT metabolomic test, developed to detect subjects with impaired glucose tolerance [22], was confirmed to be associated with 2 h-glucose and was increased mainly in CL3 and CL4, but the baseline values were not predictive of the reduction of AUC-G (Table S11).

The strength of this study lies in the use of unsupervised and supervised machine learning techniques to establish the predictive value of pre-intervention metabolic profile on the improvement in glucose metabolism in two independent cohorts and in response to lifestyle intervention (DEXLIFE study) or spontaneous weight loss (EGIR-RISC study).

Nevertheless, this study presents some limitations. The intervention length of 12 weeks in the DEXLIFE study might not be sufficient to obtain stable metabolic effects and we do not have follow up data to evaluate if improvement in glucose metabolism is preserved after the end of the intervention. However, the results were reproducible also in other conditions, i.e., weight loss not induced by a controlled lifestyle intervention, as in the EGIR-RISC study. Another limitation is the lack of a direct measurement of β-cell secretion or insulin clearance since C-peptide was not measured in the DEXLIFE study. However, C-peptide was available in the EGIR-RISC cohort and the analyses conducted showed that C-peptide OGTT response in the four clusters was similar to insulin response indicating cluster reliability with both hormones (Fig. S9). Moreover, the possible influence of other comorbidities was not considered in the analysis of DEXLIFE cohort, but considering that the validation study, the EGIR-RISC, was free of comorbidities at the time of enrollment, we can say that this has little or no effect on the results. Lastly, the performances observed by supervised modeling indicate the moderate discriminative ability but suggest room for improvement, that can be explored in future studies.

In conclusion, inter-individual variability in improvement in glucose metabolism after weight loss can be explained and predicted by pre-intervention values of insulin sensitivity, glucose-stimulated insulin response and β-cell function. Machine learning models, both descriptive and predictive, identified the pre-intervention phenotypes with the greatest glucose metabolic benefit after weight loss with non-pharmacological intervention. These findings could help clinicians identify patients with best improvement in glucose tolerance in response to lifestyle intervention or weight loss, namely those belonging to CL3 and CL4, i.e., subjects with preserved and high capacity of β-cells insulin secretion despite high insulin resistance, i.e., with high baseline OGTT-insulin concentrations.

CRediT authorship contribution statement

Silvia Sabatini: Writing – review & editing, Writing – original draft, Visualization, Software, Investigation. **John J. Nolan:** Writing – review & editing, Conceptualization. **Grainne O'Donoghue:** Writing – review & editing, Data curation. **Aileen Kennedy:** Writing – review & editing, Data curation. **John Petrie:** Writing – review & editing, Data curation. **Mark Walker:** Writing – review & editing, Data curation. **Donal J. O'Gorman:** Writing – review & editing, Conceptualization. **Amalia Gastaldelli:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Amalia Gastaldelli reports financial support was provided by European Union. John Nolan reports financial support was provided by European Union. Donal O'Gorman reports financial support was provided by European Union. John Petrie reports financial support was provided by European Union. Mark Walker reports financial support was provided by European Union. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that support the study findings are available upon reasonable request from the corresponding author. R Scripts for machine learning and statistical analysis can be downloaded from https://github.com/Silvia410/Project-DEXLIFE.

Acknowledgment

AG is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and

the accuracy of the data analysis.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.metabol.2024.155910.

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