

Prediabetes subtype stratification by β -cell function: Evidence from hyperglycaemic clamp and ectopic pancreatic–hepatic fat

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Research Article

Keywords: Prediabetes, Subtypes, Hyperglycaemic Clamp, Pancreas Imaging, Stratification

Posted Date: June 5th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9907316/v1>

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Additional Declarations: The authors declare no competing interests.

Abstract

An accurate subtype stratification of prediabetes (PD) based on insulin secretion capacity is needed for precision in its treatment and the prevention of its progression. We performed hyperglycaemic (HG) clamps to measure insulin secretion and Magnetic Resonance Imaging (MRI) of the pancreas and liver to measure their fat content, in a cohort of 255 young adults aged 18–45 years; 75 were normoglycemic (NG), and 180 had PD. K-means clustering of the baseline HOMA-B (Homeostatic Model Assessment of β -cell function) and plasma C-peptide concentrations in the PD group identified a low insulin-secreting (insulin deficient-ID subtype, 77.1%), and high insulin-secreting (insulin resistant-IR subtype, 22.9%) subtypes, with a robust clustering performance and stability (silhouette score = 0.52, Rand index = 0.95, Hopkins statistic = 0.97, and mean Jaccard similarity = 0.93). HG clamp indices confirmed significantly lower 1st and 2nd phase insulin secretion responses, with higher insulin sensitivity and hepatic clearance, in the ID compared to the NG and IR subtype. There was significantly higher mean ectopic fat deposition in liver and pancreatic tail, in the IR compared to ID subtype. Hepatic and pancreatic fat correlated negatively with insulin sensitivity and positively with basal insulin levels across the cohort. Further, pancreatic signal intensity ratio negatively correlated with insulin secretion and positively with insulin sensitivity in NG and IR subtype. An analysis of biomarkers to discriminate the ID and IR subtypes of PD identified the C-peptide index as a potential discriminatory biomarker, with a median AUC of 0.988 and accuracy of 94%.

Introduction

Type 2 diabetes (T2D) is a highly prevalent and biologically heterogeneous disease with major global health consequences^{1,2,3}. Despite advances in its management over the past two decades, prevention and treatment strategies remain largely uniform and do not account for underlying biological heterogeneity⁴. Recent efforts to classify T2D into distinct subtypes have revealed important differences in genetic architecture, aetiology, pathophysiology, clinical trajectories, complications, and therapeutic responses^{2,5–11}. However, these approaches have largely focused on individuals with established diabetes, whereas data on heterogeneity at earlier stages of dysglycaemia, particularly prediabetes (PD) remains scant¹².

PD, currently characterized by impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT), affects a substantially larger population than overt T2D, and its burden is increasing rapidly in low- and middle-income countries^{13,14}. IFG and IGT are known to be caused due to impaired sensitivity (hepatic or peripheral) and/or impaired secretion (basal or post-prandial) leading to elevated levels of HbA1c^{15,16}. Some populations, such as South Asians, exhibit distinct metabolic phenotypes along with rapid progression from PD to T2D^{17–19}. Together, these observations underscore the need to identify clinically relevant PD subtypes for earlier risk stratification that would result in targeted treatments and progression prevention strategies.

Although some studies have proposed subtypes of PD, typically reflecting differences in insulin deficiency versus insulin resistance, these classifications have largely relied on surrogate indices with limited physiological precision^{20,21}. As a result, the biological validity of these proposed subtypes remains uncertain. We sought to characterize PD subtypes in a screening-based cohort of 255 young individuals, aged 18–45 years without T2D, using robust physiological measurements of insulin secretion and insulin resistance. We did this by measuring their insulin and C-peptide secretion capacity using the hyperglycaemic clamp (HG clamp)²² technique and compared their profiles to those with normoglycemia (NG). Furthermore, we integrated magnetic resonance imaging proton density fat fraction (MRI-PDFF) based phenotyping in these individuals to assess the pancreatic and hepatic tissue ectopic fat deposition across subtypes. By combining detailed HG clamp-based physiological and MRI-based imaging characterization, this study provides a mechanistically grounded framework for subtyping PD in Indian participants.

Results

The cohort: The participant recruitment is shown in Extended Figure1 (online methods). Their demographic and clinical data are provided in Suppl Table1. Briefly, through screening of 8500 individuals with body mass index (BMI) <25 kg/m² and age between 18-45 years, 2383 individuals were selected for assessment of glycaemic status using their plasma glycated Hb concentration (HbA1c). Using this criterion, we recruited 255 participants (48 females and 207 males) of whom 75 were NG with HbA1c <5.7%, and 180 had PD with HbA1c between 5.7-6.4% following ADA guidelines 2025²³. The distribution of HbA1c across these participants is shown in Suppl Figure1. The median age of participants was 29 years (75% IQR 24-35) for the NG group and 26 years (75% IQR 23-33) for the PD group, with no significant differences in age or BMI between the two groups. The recruited participants underwent a 2-hour HG clamp study²².

Cluster analysis revealed two distinct PD subtypes

Of the 255 participants recruited for the HG clamp studies, 7 in the NG group were excluded due to technical difficulties with venous cannulation. The clamp output data were pre-processed for the removal of outliers, using the median absolute deviation-based threshold, which resulted in the availability of 175 PD participants for further analysis. An exploratory k-means clustering analysis was performed using fasting plasma glucose, HbA1c, insulin, and C-peptide concentrations, age, BMI, HOMA-IR and HOMA-B, to identify the best variables that yielded a better silhouette score, such that stable clusters could be assessed by various clustering diagnostic parameters (Suppl Table 2a). The cluster analysis among participants with PD, based on basal HOMA-B and basal plasma C-peptide concentrations, revealed two distinct clusters with silhouette score of 0.52, Rand index of 0.95, Hopkins statistics value of 0.97 and mean Jaccard similarity index of 0.93. The cluster variables had a modest Spearman correlation of 0.547 and variance inflation factor of 1.46 with the dominant PC1 axis that explained 78% of the variance. Since insulin secretion and sensitivity are mechanistically related, the

cluster diagnostics suggested that these clusters may not be orthogonal subtypes but represent a 1-D gradient with lower values representing lower insulin secretion (or β -cell function) and higher values representing higher secretion. One cluster, characterized as low insulin-secreting, was called the insulin deficient (ID) subtype (n=135, 77.1%), and the second cluster, or the high insulin-secreting group, was called the insulin resistant (IR) subtype (n=40, 22.9%) (Figure 1a).

Stability of the ID and IR subtypes as assessed by HG clamp outputs

To assess the stability of the subtype clusters obtained from HOMA-B and basal plasma C-peptide concentration, we compared their performance with clusters obtained using HG clamp outputs. For the clusters to be comparable, while clustering on HG clamp measures, either HOMA-B or Insulin Secretion Rate (ISR), that define β -cell function, were retained as one of the variables. Results of cluster performance along with the percent similarity and error rates for each of the cluster comparisons are reported in Suppl Table 2b. The similarity of clusters obtained using HOMA-B and AUCs (area under the curve) of 1st phase and 2nd phase C-peptide response and ISR, and mean plasma C-peptide and insulin concentrations, and mean ISR, is shown in Figure 1b & 1c.

For the 1st phase plasma C-peptide response, the cluster similarity with the ID cluster was 95.3% and with the IR cluster (obtained by basal features) was 75.6%, whereas for the 1st phase insulin response, it was 92.4% with the ID and 71.8% with the IR cluster, with an overall error rate of ~10-15% for both 1st phase secretions. For the 2nd phase C-peptide response, the cluster similarity was 94.21% with the ID and 65% with the IR cluster, whereas for 2nd phase insulin secretion, it was 92.2% with ID and 69% with IR cluster with an overall error rate of ~15% for both 2nd phase secretions. On clustering with the mean (0-120) plasma insulin and C-peptide concentrations, the ID cluster was 92.13% and 92.7% similar respectively, while the IR cluster was 65% and 81% similar respectively, with an overall error rate of 16-17%. Clustering on 1st and 2nd phase ISR and mean ISR with HOMA -B, the ID cluster was 94-97% similar, whereas the IR cluster was around 70% similar with an error rate of 12% and 14% for the 1st phase ISR and mean ISR, respectively. The clusters obtained by either 1st phase ISR or mean ISR with the 1st phase, 2nd phase and mean insulin or C-peptide secretions showed ~ 85 % similarity with the ID cluster and 60-75% similarity with the IR cluster, with an error rate of ~18-21% with different secretion measures (Suppl Table 2b).

When clustering on HG clamp based measures (Suppl Table2b), it was observed that some individuals in the IR subtype (i.e.~3-6% of overall PD (n=5-10) slid into the ID subtype, whereas some individuals of the ID subtype (i.e.~6-10% of overall PD (n=10-16) slid into the IR subtype, suggesting intra-individual heterogeneity in β -cell function at the basal and hyperglycaemic clamped state. The sliding of the individuals between the clusters when clustered on HG clamp variables is represented by their respective colours in the Figure 1 b-d. Those individuals who were classified as ID on basal measures but slid into the IR subtype on clustering with HG clamp measures, had lower HOMA-IR and HOMA-B at baseline; with ~30-40% increase in mean ISR and ~35-45% lower Insulin Sensitivity Index (ISI) when HG clamped, when compared to the NG group. On the other hand, those individuals classified as IR on basal measures, but

who slid into the ID group on clustering with HG clamp outputs, had a higher HOMA-IR and HOMA-B at baseline; however, with ~25-30% lower mean ISR and ISI at par with the NG group.

Demographic and clinical characteristics of NG group and PD subtypes

Demographic and clinical characteristics of IR and ID clusters are summarized in Table 1.

Notably, the mean age, BMI, waist and hip circumferences and plasma lipid concentrations were lower in the ID cluster compared with IR cluster, and similar to the NG cluster (Figure 2).

Table 1: Demographic and clinical characteristics of PD subtypes

Variable	NG (n = 68)	Prediabetes		p value [#]	p value ^{\$}		
		IR (n = 40)	ID (n =135)		NG vs IR	NG vs ID	IR vs ID
Demographics							
Age (year)	29.0 (24.0-35.0)	27.0 (24.0-34.0)	25.5 (23.0-32.0)	0.046	0.922	0.084	0.127
BMI (kg/m ²)	22.4 (20.5-23.4)	22.8 (22.1-24.1)	21.7 (20.0-23.6)	0.040	0.038	0.311	0.027
Waist (cm)	82.0 (77.7-88.3)	84.0 (82.0-88.0)	81.0 (76.0-85.0)	0.020	0.356	0.147	0.025
Hip (cm)	96.0 (91.0-99.3)	95.0 (91.0-98.0)	93.0 (89.3-97.0)	0.016	0.940	0.035	0.065
W/H	0.9 (0.8-0.9)	0.9 (0.8-0.9)	0.9 (0.8-0.9)	0.189	0.454	0.427	0.356
HbA1c (%)	5.4 (5.2-5.6)	5.9 (5.8-6.0)	5.9 (5.8-6.0)	< 0.001	< 0.001	< 0.001	0.396
Hemoglobin (g/dL)	14.1 (12.6-15.2)	14.5 (13.3-15.3)	14.4 (13.2-14.9)	0.786	0.757	0.757	0.757
Liver function test							
Total Bilirubin mg/dL	0.7 (0.6-1.0)	0.7 (0.5-0.8)	0.7 (0.5-0.9)	0.716	0.719	0.988	0.719
Direct Bilirubin (mg/dL)	0.2 (0.10-0.2)	0.1 (0.1-0.2)	0.10 (0.10-0.2)	0.369	0.434	0.434	0.434
Indirect Bilirubin (mg/dL)	0.6 (0.4-0.8)	0.5 (0.4-0.7)	0.6 (0.4-0.8)	0.767	0.749	0.749	0.749
SGPT (ALT) (U/L)	20.0 (14.0-30.3)	25.0 (16.3-33.0)	19.0 (14.0-27.0)	0.129	0.108	0.951	0.108
SGOT(AST) (U/L)	21.5 (18.0-27.3)	22.0 (19.0-27.0)	24.0 (20.0-28.0)	0.074	0.813	0.106	0.228
ALP (U/L)	72.5 (61.3-89.0)	71.5 (66.0-85.0)	78.0 (66.0-95.0)	0.064	0.813	0.093	0.237
Total Proteins (g/dL)	7.3 (7.0-7.6)	7.4 (7.2-7.7)	7.3 (7.0-7.6)	0.061	0.047	0.852	0.047
Albumin (g/dL)	4.4 (4.2-4.6)	4.5 (4.4-4.7)	4.4 (4.2-4.6)	0.023	0.024	0.479	0.029
Globulin (g/dL)	2.9 (2.6-3.10)	2.9 (2.7-3.3)	2.9 (2.6-3.10)	0.532	0.669	0.724	0.669

A/G ratio	1.5 (1.4-1.7)	1.6 (1.4-1.7)	1.55 (1.4-1.7)	0.823	0.890	0.890	0.890
Renal Function test							
Blood Urea (mg/dL)	21.0 (18.0-25.5)	21.0 (17.0-24.7)	20.0 (17.0-23.0)	0.054	0.478	0.055	0.377
Serum Creatinine (mg/dL)	0.8 (0.7-1.0)	0.9 (0.8-1.0)	0.9 (0.8-0.9)	0.475	0.515	0.636	0.515
Sodium (mEq/L)	138.0 (137.0-139.0)	138.0 (137.0-139.0)	138.0 (137.0-139.0)	0.521	0.440	0.925	0.440
Potassium (mEq/L)	4.10 (3.9-4.3)	4.2 (4.0-4.3)	4.2 (4.0-4.4)	0.105	0.275	0.108	0.869
Chloride (mEq/L)	104.0 (102.5-106.0)	103.0 (102.0-104.7)	104.0 (102.0-105.0)	0.217	0.320	0.326	0.326

Values were presented as median (IQR), where IQR denotes the interquartile range (Q1–Q3).

Indicates overall intragroup comparisons between variables using the Kruskal–Wallis test.

§ Indicates intergroup comparisons between variables using the Kruskal–Wallis test followed by Dunn’s post hoc test with Benjamini–Hochberg (BH) adjustment

SGOT: Serum Glutamic-Oxaloacetic Transaminase; AST: Aspartate Aminotransferase

SGPT: Serum Glutamic Pyruvic Transaminase; ALT: Alanine Aminotransferase

ALP: Alkaline Phosphatase

Insulin, C-peptide and HOMA indices indicate higher insulin sensitivity and hepatic clearance in ID subtype of PD

Insulin and C-peptide responses in the subtypes across 17 time points during the 2-hour clamp are shown in (Figure 3a). Basal plasma insulin and C-peptide concentrations, HOMA indices including HOMA-IR, HOMA-B, HOMA2 and 1st phase, 2nd phase ISR and C-peptide secretion during the HG clamp were significantly higher ($P<0.001$) in IR cluster compared to NG and ID cluster. In contrast, insulin sensitivity, basal and steady state hepatic insulin clearance were significantly higher in the ID than the IR subtype and NG group ($P<0.001$). The HG-clamp based 1st and 2nd phase ISR were lower, and the ISI was higher ($P<0.001$), in the ID compared to IR subtype and the NG group. The ID cluster was composed of ~92% individuals with HOMA-B<100, and ~78% individuals with HOMA-IR<2, whereas the IR cluster was composed of 90% individuals with HOMA-B>100 and HOMA-IR>2. Although the disposition index (DI) is the gold standard to discriminate subtypes in diabetes, it was not statistically different between the subtypes in our prediabetes cohort. The forest plot shows (Figure 3b) the median fold change in several basal and clamp-based secretion and sensitivity measures with significance values in ID and IR

subgroups is shown in Suppl Table 3. Figure 3c represents the phenotype coherence by basal and HG clamp-based measures in ID and IR subtype differentiated by the median of NG groups.

Diagnostic performance of a potential biomarker to discriminate PD subtypes

To investigate potential biomarkers for discriminating the PD subtypes (ID or IR), we tested the diagnostic accuracy of several basal and HG-clamp based secretion measures and indices using Receiver Operating Characteristic (ROC) analysis. This analysis (using cluster-identified subtypes as outcome) was performed by bootstrapping 1,000 iterations to assess robustness of biomarker performance (Figure 4) and Youden cut-off values. In the basal measures, the C-peptide index demonstrated the highest discriminatory performance with median test AUC of 0.988 (95% CI: 0.97-1.00), cut-off 0.439, sensitivity 100.0% (95% CI: 91.7-100.0%), specificity 92.7% (95% CI: 85.4-97.6%), and accuracy 94.3% (95% CI: 88.7-98.1%), indicating an outstanding separation between the PD subtypes. This was followed by HOMA-B and HOMA2-B (AUC 0.982), Insulin (AUC 0.978), HOMA2-S (AUC 0.967), HOMA-IR (AUC 0.953) and C-peptide (AUC 0.949). Among the HG-clamp based measures, the 1st Phase ISR demonstrated the highest discrimination with a test AUC 0.842 (95% CI: 0.73-0.94), cut-off 1156.24 (95% CI: 1154.89-1332.22), sensitivity 83.3% (95% CI: 50.0-100.0%), specificity 75.0% (95% CI: 62.5-87.5%), and accuracy 76.9% (95% CI: 66.7-87.2%). This was followed by ISI with an AUC 0.827 (95% CI: 0.72-0.92), cut-off 4.94 (95% CI: 4.84-6.12), sensitivity 83.3% (95% CI: 58.3-100.0%), specificity 70.7% (95% CI: 48.8-82.9%), and accuracy 73.6% (95% CI: 58.5-83.3%), 1st phase C-peptide response (AUC 0.823), mean C-peptide (AUC 0.789), mean ISR (AUC 0.783). The details of the cut-off values, test AUCs, sensitivity, specificity and accuracy, along with their bootstrapped confidence intervals for the biomarkers tested, are reported in (Suppl Table 4). Narrow confidence bands (shaded regions in ROC) indicate stable performance with minimal overfitting and robustness of the biomarkers, with the C-peptide Index demonstrating the best test performance.

We further tested the diagnostic performance of the optimal cut-off value of the C-peptide index (0.44) to classify low vs high HOMA-B, HOMA-IR, ISR and ISI phenotypes using the median of these parameter distributions in the NG subtype to define their cut-offs. The diagnostic performance of C-peptide index was an AUC of 0.89 (95% CI: 0.81 - 0.95) for HOMA-B, AUC of 0.87 (95% CI: 0.81-0.92) for HOMA-IR, AUC of 0.8 (95% CI: 0.74-0.87) for ISR and 0.84 (95% CI: 0.78-0.90) for ISI, suggesting a good performance in detecting both phenotypes related to insulin secretion and sensitivity on the basal and HG clamp measures (Figure 4c). On further stratifying the PD subjects with C-peptide index above the cut-off value as high insulin secreting but lower sensitivity individuals (high HOMA-B - high HOMA-IR or high ISR - Low ISI: a surrogate of the IR subtype) and below the cut-off values as low insulin secreting but higher sensitivity individuals (low HOMA-B - low HOMA-IR or low ISR - high ISI: a surrogate of the ID subtype), the diagnostic performance of the C-peptide index was excellent, with AUC of 0.98 (95% CI: 0.96-0.99) and accuracy of 0.93 for basal and AUC of 0.9 (95% CI: 0.85-0.95) and accuracy of 0.84 for clamp measures (Figure 4d).

Magnetic resonance imaging and FibroScan reveal significant differences in ectopic fat in PD subtypes

Hepatic fat (HF) measured using either MRI-PDFF (proton density fat fraction) or FibroScan CAP value (controlled attenuation parameter) was significantly higher in the IR subtype ($p=0.002$ by MRI, $p<0.001$ by CAP) and lower in the ID subtype, similar to the NG group. Pancreatic fat (PF, measured by PDFF) trended higher in the IR subtype, especially in the tail region, but not significantly so ($p=0.324$). Pancreas volume based on measurements using T1w MRI was slightly lower in both subtypes and T2w MRI was lower (but not significant) in the ID subtype compared to the NG group. Pancreas T2 relaxation time was significantly higher in the ID subtype in comparison to the NG subtype ($p=0.048$). Pancreas T1 signal intensity in the tail region was lower in both prediabetes subtypes and was particularly significant in IR subtype ($p=0.035$). Details of differences in MRI features among the groups are shown in Suppl Table 5 and Figure 5 A.

MRI measures correlate with HG clamp-based indices

We performed correlation analyses for the basal and HG clamp output variables with MRI measures of hepatic fat, pancreatic fat, pancreas relaxation time T2 (PRT2) and pancreas signal intensity ratio (PSIR) to assess the associations within the phenotypes. All the statistically significant correlations with $p<0.05$ and False Detection Rate (FDR) <0.1 are shown in Figure 5. The detailed p-values and FDR values for the correlations are reported in Suppl Table 6 (Excel file)

In the NG group, HF and PF were positively correlated with fasting insulin ($\rho=0.42$, $p<0.001$), C-peptide ($\rho=0.36$, $p=0.003$), HOMA-IR ($\rho=0.42$, $p<0.001$), HOMA-B ($\rho=0.35$, $p=0.004$), 1st phase insulin secretion ($\rho=0.29$, $p=0.019$) and C-peptide secretion ($\rho=0.33$, $p=0.007$), and 1st and 2nd phase ISR ($\rho=0.32-0.41$, $p=0.001-0.012$). HF was also positively associated with 2nd phase insulin, C-peptide, and ISR ($\rho=0.26-0.38$, $p=0.005-0.05$). Both HF and PF were inversely correlated with QUICKI ($\rho=-0.42$ and -0.40 , respectively; $p\leq0.001$), ISI ($\rho=-0.44$, $p<0.001$), and insulin clearance measures, including hepatic insulin clearance ($\rho=-0.27$, $p=0.028$) and HG clamp-derived insulin clearance ($\rho=-0.29$, $p=0.03$). In the IR subtype, PF showed positive correlations with fasting insulin ($\rho=0.30$, $p=0.056$), HOMA-IR ($\rho=0.29$, $p=0.072$), and HbA1c ($\rho=0.31$, $p=0.048$), while HF was positively correlated with C-peptide ($\rho=0.34$, $p=0.036$) and showed trends toward inverse associations with ISI ($\rho=-0.26$, $p=0.104$) and DI ($\rho=-0.24$, $p=0.134$). However, these associations did not remain significant after FDR correction (FDR >0.1). In the ID subtype, PF was positively correlated with plasma glucose, insulin, C-peptide, HOMA-IR, and HOMA-B ($\rho=0.25-0.35$, $p<0.001-0.012$). Both HF and PF were inversely associated with ISI (HF: $\rho=-0.27$, $p=0.002$; PF: $\rho=-0.17$, $p=0.057$), DI (HF: $\rho=-0.25$, $p=0.005$; PF: $\rho=-0.19$, $p=0.037$), and insulin clearance (HF: $\rho=-0.19$, $p=0.027$). PF also showed a negative correlation with QUICKI ($\rho=-0.26$, $p=0.03$).

In the NG group, and IR subtype PSIR in the tail region although trended towards correlations, did not survive after FDR correction. However, In the ID subtype, PSIR in the pancreatic tail was positively

associated with fasting glucose, basal C-peptide, 1st phase insulin and C-peptide secretion, and 2nd phase ISR ($p=0.20-0.23$, $p=0.01-0.05$).

No significant correlations were observed between pancreatic T2 relaxation time (PRT-T2) and metabolic indices in the NG group. In the IR subtype, PRT-T2 in the pancreatic head showed a negative correlation with basal C-peptide ($p=-0.36$, $p=0.038$) and PRT-T2 in the tail showed a positive correlation with fasting glucose ($p=0.37$, $p=0.048$), although both associations had $FDR>0.1$. In the ID subtype, PRT-T2 in the head showed a trend toward a negative association with 1st phase C-peptide secretion ($p=-0.15$, $p=0.110$; $FDR>0.1$).

Discussion

Our study, using HG clamps in young, non-obese, participants without T2D ($n = 255$) aged 18–45 years, identified two distinct PD clusters or subtypes based on β -cell function and insulin sensitivity. In this population of young adults with BMI ranging from 18–25, ~ 75% of PD was due to insulin deficiency (ID) and 25% was due to insulin resistance (IR). Interestingly, the ID subtype had a higher ISI and a higher hepatic insulin clearance, despite lower β -cell function, while the IR subtype had a low insulin sensitivity and hepatic clearance with higher β -cell function. Additionally, we imaged the hepatic and pancreatic fat, along with other parameters, and found significant correlations with HG clamp indices. For example, hepatic fat was higher in the IR subtype and was positively correlated with insulin indices. Similarly, pancreatic fat was higher in IR, while the pancreatic T2 relaxation time was higher in ID subtype on MRI, reflecting differences in fat content and inflammation between the two. Among the various clinically actionable biomarkers that were evaluated to discriminate PD subtypes, the C-peptide index was the most accurate, offering a feasible and practical clinical tool for the precision management of PD.

The demonstration of the ID and IR clusters using HOMA-B and C-peptide, corroborates with other studies that subtyped diabetes and PD in South Asians.^{5,20,24,25} These prior studies relied primarily on demographic variables and the HOMA indices for their cluster analyses. However, some identified more clusters; for example, Zheng et al identified six PD clusters in Chinese individuals of a range of BMI, using baseline BMI, WHR, HOMA indices, triglycerides, fasting glucose, HbA1c, ALT, AST and GGT, as well as follow-up for diabetes complications²¹. Among their six clusters, 39.4%-64.3% of PD individuals with BMI ranging from 21.5–24.1 kg/m² progressed to severe insulin deficient T2DM, indicating that a high proportion of low BMI PD individuals progress to severe insulin deficient diabetes (SIDD)²¹. Our investigation incorporated a validation and stability of defined PD clusters using the HG clamp indices and C-peptide, in addition to the standard HOMA-B method. Our study also aligns with the published literature reiterating higher prevalence of ID in prediabetes^{20,26}, along with ethnic variation in the frequency of this phenotype. The uniqueness of our study is that we included young and normal BMI individuals with PD, demonstrating that ~ 75% of them had low insulin secretion (ID) revealing inherent early β -cell dysfunction. This is relevant, as the CARRS cohort study found a higher mortality risk in its ID group²⁰ highlighting the importance of identifying this subtype at the PD stage, even in normal BMI

individuals, to potentially prevent progression to diabetes, avert related complications and prevent mortality.

We used basal clinical measures (HOMA-B and C-peptide) for our clustering exercise, as these can be easily translated to clinics. While the ID subtype was composed of low secreting individuals the IR subtype included only high secreting but IR individuals. These clusters are clinically relevant as treatments can be tailored to address either the secretion defect or insulin resistance at the prediabetic stage. The 10–20% misclassification rate using HG clamp measures may be attributed to the inherent heterogeneity in the physiological regulation of fasting plasma glucose concentrations and glucose tolerance, as they have different metabolic characteristics²⁷. Per previous reports, while isolated IFG or IGT show a similar reduction in the 1st phase insulin response, IFG is mainly associated with impaired basal insulin secretion and preferentially hepatic IR, while IGT is mainly associated with reduced 2nd phase insulin secretion and peripheral IR^{28,29}. In our data, the sliding of 5–10% individuals between clusters (because of misclassification on HG clamp output) was mainly due to differences in ISR and ISI that were in contrast to their assigned group based on basal measures, indicating a mixed phenotype.

We evaluated β cell function and insulin sensitivity to define the PD subtypes by assessing basal, 1st and 2nd phase plasma insulin and C-peptide responses during the HG clamp. HOMA indices, HOMA-2 indices and clamp-based indices (C-peptide index), QUICKI, and the hepatic clearance index point to a higher state of β -cell function and lower insulin sensitivity in the IR subtype, and significantly lower state of β -cell function and higher insulin sensitivity in the ID subtype. Our study did not find any difference in the DI in either subtype, contrary to earlier reports which suggested that the DI might be a suitable gold standard to discriminate subtypes of diabetes^{30,31}. Since DI is the product of ISI and ISR, a lower ISI could be compensated by a higher ISR in the IR subtype and vice-versa in the ID subtype, clearly indicating that the DI could be a poor discriminator of PD subtypes in young and normal BMI individuals, as shown in this study.

We evaluated the diagnostic performance of basal and HG clamp-based indices to identify potential biomarkers to discriminate PD subtypes. From ROC analyses, we found that the basal C-peptide index discriminated these subtypes with median AUC of 0.988 and 94% accuracy, whereas the 1st phase ISR was the best performing HG clamp-based measure with AUC of 0.842 with 77% accuracy. Both C-peptide index and 1st phase ISR would be clinically relevant indices as they both represent β -cell function and can assess early β -cell failure in PD. The diagnostic performance of C-peptide index (AUC of ~ 0.85) in discriminating high vs low ISR or ISI further supports its diagnostic potential in identifying ID and IR subtypes of PD in routine clinical practice. To our knowledge, this is the first report indicating the discriminatory importance of C-peptide in ID and IR subtypes of PD.

Based on available literature, this is the first study to correlate liver and pancreatic imaging findings with HG clamp indices to elucidate the pathophysiology of prediabetes. The significantly higher HF in the IR subtype, demonstrated by both imaging modalities, reinforces its role in insulin resistance. In contrast, PF in the ID subtype was associated with lower ISI, DI, and higher plasma glucose levels despite normal

BMI, suggesting a distinct pathogenic mechanism. In the NG and IR groups, HF and PF were associated with enhanced β -cell secretion alongside reduced insulin sensitivity, consistent with a compensatory response. Across the cohort, HF was inversely associated with insulin clearance, supporting its role in modulating insulin metabolism. Correspondingly, the ID subtype, characterized by low HF and higher insulin sensitivity, exhibited increased hepatic insulin clearance, whereas the IR subtype showed the opposite pattern. The presence of isolated pancreatic fat accumulation in the ID subtype, in the absence of increased HF, suggests an intrinsic islet cell defect contributing to its pathophysiology.

Beyond differences in HF and PF content, pancreatic volume, relaxation time, and signal intensity ratios differed between the subtypes. Both PD subtypes exhibited lower pancreatic volume than the NG group is consistent with reports of early pancreatic atrophy in type 1 and type 2 diabetes^{34–37}, our data shows lowest pancreatic volume in the ID subtype. Despite reduced volume, pancreatic fat content was higher in both PD subtypes, with the greatest accumulation in the pancreatic tail of the IR subtype, suggesting that pancreatic fat deposition may precede overt diabetes, particularly in insulin-resistant individuals.

Lower PSIR in both prediabetes subtypes, particularly in the pancreatic tail of IR subtype, reflects increased pancreatic fat deposition. Its association with insulin secretion indices suggests a compensatory β -cell response to reduced insulin sensitivity in the IR and NG groups, indicative of preserved β -cell function^{38,39}. In contrast, the positive association of pancreatic tail PSIR with fasting glucose, basal C-peptide, and insulin secretion rate in the insulin-deficient (ID) subtype suggests impaired β -cell secretory capacity, possibly related to acinar–islet remodelling associated with ectopic fat accumulation^{40–43}. The higher pancreatic T2 relaxation time in the ID subtype suggests altered tissue composition, including inflammation and fibrosis⁴⁴. Its negative association with C-peptide secretion further supports a link between pancreatic structural changes and impaired insulin secretory function.

Our study has several strengths, including the conduct of HG clamps in a large cohort, the identification and validation of PD subtype clusters at basal and steady state maximal insulin secretion, the identification of a potential diagnostic biomarker to distinguish ID and IR phenotypes in PD, and a comprehensive correlational analysis of HG clamp indices with imaged determinations of fat content in liver and pancreatic tissues. One possible limitation of our study is that we used HbA1c to categorize participants into NG and PD, as in previous studies⁷, irrespective of their glycaemic status, as HbA1c is a stable marker of long-term glucose levels. This was because we were screening at scale, and conducting the oral glucose tolerance test (OGTT) for screening and the HG clamp in included participants on consecutive days was challenging. Since anaemia was an exclusion criterion in this study, the use of HbA1c would not lead to an overestimation of PD, and the composition of our cohort, of ~ 25% IR and ~ 75% ID individuals, agrees with previous reports that suggest a 30:70 respective contribution of IFG and IGT towards HbA1c in PD⁴⁵.

In conclusion, HG clamp outputs identified the ID subtype as the predominant PD phenotype among South Asian nondiabetic individuals, indicating inherent β -cell dysfunction, even at a young age, despite normal BMI. Ectopic fat deposition in the liver and pancreas, along with reduced signal intensity on MRI

in the IR phenotype, reflects compromised pancreatic tissue health and suggests the presence of subclinical metabolic alterations prior to the onset of IR subtype of prediabetes. These studies indicate the need to assess whether underlying pancreatic islet structure, function, and cell composition are responsible for the reduced insulin secretion, or whether circulating metabolic factors are responsible. Finally, we highlight the C-peptide index as a promising discriminative diagnostic marker for PD subtypes, enabling targeted screening and personalized intervention approaches.

Declarations

Acknowledgements:

MS, AVK, PRS, KMV and ACP thank the India Alliance (DBT Wellcome Trust) for financial support through a Clinical Research Center grant (IA/CRC/23/1/600505) to establish the Asian Centre for Endocrine Pancreas Research for the study of type2 diabetes in India. The investigators would also like to thank the advisory board members for their valuable support and scientific suggestions. The authors express their gratitude to all the participants for consenting to be part of the study. Additionally, ACP is supported by DK020593 (Vanderbilt Diabetes Research and Training Center and the Department of Veterans Affairs (BX000666). We thank V. Sai Sarada for assistance with the preparation of the basic statistical tables used in the study.

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Author contribution:

A.V.K conceptualized and designed the clamp study and trained the staff at Asian Centre for Endocrine Pancreas on Hyperglycemic clamp along with S.K. and supervised the clamp procedures, Data interpretation, manuscript review and editing. A.V.K is the corresponding author. M.S conceived and designed the study, supervised the clamp, project administration, oversaw its implementation, wrote the original draft of the manuscript, and finalized. V.N, D.S conducted HG Clamp along with B.P.R and N.V and participated in the maintenance of the database. V.N was involved in the analysis of data, drafting the manuscript along with M.S. B.P.R., N.V were involved in conducting the clamp. P.S.S was involved in screening, participant recruitment and project management. R.S.K was involved in coordinating with the participants, taking consent and filling lifestyle and nutrition questionnaire. V.V.K was involved in sample separation. T.P, S.B.D, N.S were involved in MRI hepatic-pancreatic imaging of all participants accepted for clamps. D.A.R was involved in MRI data reviewing and editing of the manuscript. L.R.S, provided valuable comments on the data analysis and edited the manuscript. J.V. was involved with MRI data review and contributed to manuscript review. A.C.P conceptualized the study, provided critical inputs and reviewed the manuscript. K.M.V was involved in design of the study, scientific input to the study, drafting and editing of the manuscript. P.R.S, performed statistical and cluster analysis of the clamp data, diagnostic biomarker analysis, interpreted and correlated clamp data with Imaging data, involved in the drafting, revision, and editing of the manuscript. K.C and T.C assisted P.R.S in statistical and correlation analysis. K.M.V and P.R.S share co-corresponding authorship. D.N.R created the facility for studies on diabetes and provided partial financial and clinical support for the study. A.V.K, M.S, K.M.V, A.C.P and P.R.S participated in discussions to assess the progress of the study regularly.

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Figures

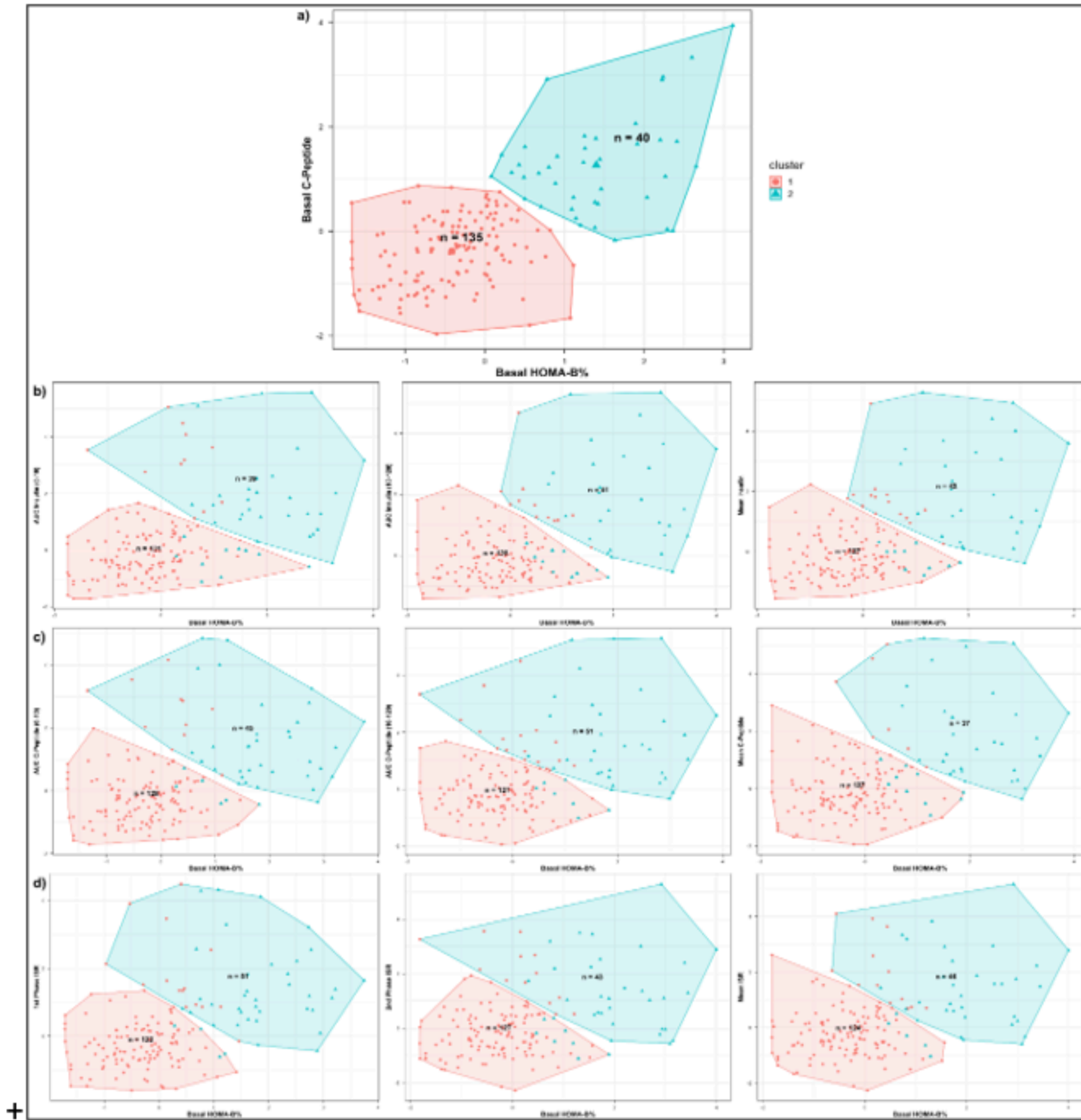


Figure 1

Cluster identification and stability

a) The plot shows two clusters obtained using basal HOMA-B and C-peptide as clustering variables; low insulin secreting (ID) and high insulin secreting subtype (IR). Each data point represents an individual observation, with the x-axis representing the basal HOMA-B and the y-axis showing the basal C-peptide (ng/ml). b) The plot shows clusters obtained for HOMA-B vs AUC of insulin-1st phase (0-10min), 2nd phase (10-120min) and mean (0-120min). The clusters are defined by the K-means algorithm, with each cluster's boundary outlined by a convex hull. c) The plot shows clusters obtained for HOMA-B vs AUC of

C-peptide-1st phase (0-10min), 2nd phase (10-120min) and mean (0-120min). d) The plot shows clusters obtained for HOMA-B vs 1st phase ISR (0-10min), 2nd phase ISR (10-120min) and mean ISR (0-120min). In Fig b, c & d, one of the variables is the basal variable (HOMA-B) and the other one is the HG clamp-based secretion variable (insulin and C-peptide). In Fig b-d, the clusters also represent the individuals swapped between the ID and IR subgroups when clustered using one of the variables as the HG-clamp based variable. The red colour dots in cyan coloured clusters indicate the sliding of individuals from ID to IR group, whereas the cyan triangles in red coloured clusters indicate vice versa.

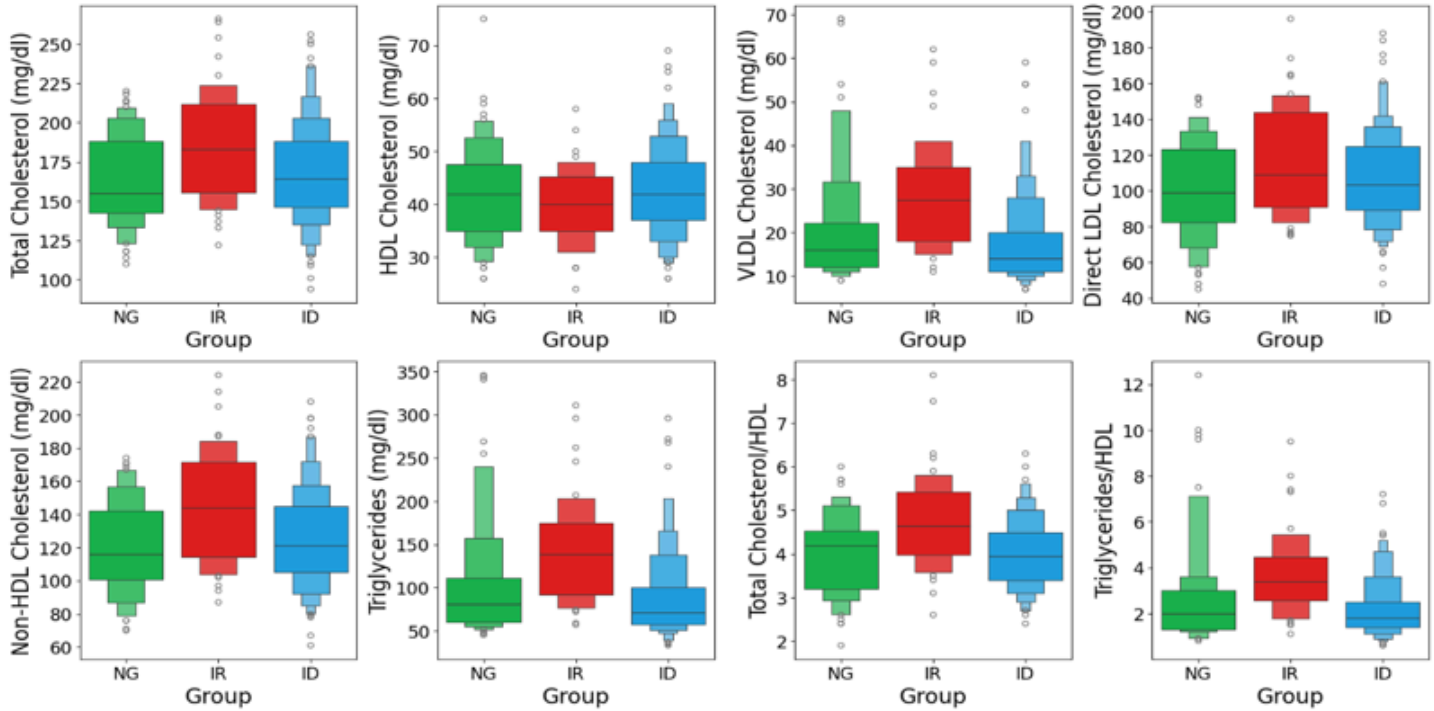


Figure 2

Lower lipid parameters in the ID compared to IR and NG group

Comparison of lipid profile parameters among NG, IR and ID subgroups. Bars represent Median (IQR) for total cholesterol (TC) ($p=0.009$), high-density lipoprotein (HDL) cholesterol ($p=0.181$), very low density lipoprotein (VLDL) cholesterol ($p<0.001$), Direct LDL cholesterol ($p=0.05$), Non-HDL cholesterol ($p=0.0092$), triglycerides (TG) ($p<0.001$), Total cholesterol/ HDL, and TG/HDL. Differences between groups were analysed using Kruskal–Wallis test with Dunn’s post-hoc analysis.

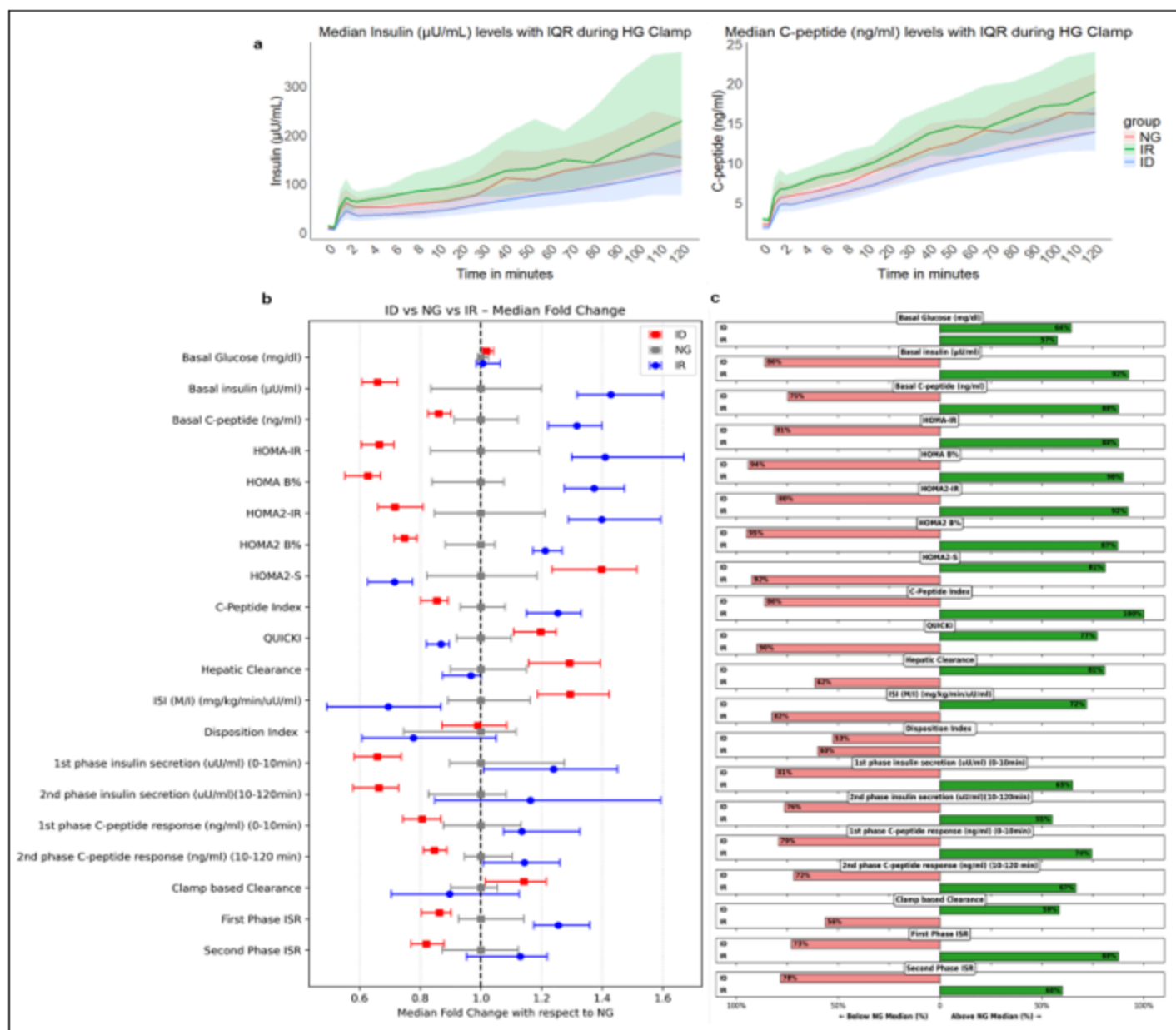


Figure 3

Clamp based indices in NG group and PD subtypes

a) Insulin response during the hyperglycaemic clamp in NG, high insulin (IR), and low insulin (ID) clusters. Insulin concentrations ($\mu\text{U/mL}$) are shown over time from baseline to 120 minutes. Data are presented as median (IQR). C-peptide response during the hyperglycaemic clamp in NG, high insulin (IR), and low insulin (ID) subgroups. C-peptide concentrations (ng/mL) are shown over time from baseline to 120 minutes. b) The forest plot representing the median fold change and their 95% confidence intervals for basal and clamp variables in ID and IR subtypes with respect to NG. Colour representation: NG-grey, ID-red and IR-blue. The plot represents all the basal secretions and sensitivity measures discriminated between IR and ID subgroups with respect to NG. HG-Clamp based 1st phase secretions of insulin and C-peptide, ISR and ISI show discrimination in ID and IR subgroups with respect to NG. c) Phenotype

coherence plot representing the proportion of individuals in each subtypes whose biomarker values are lower or higher than median values of the biomarkers in the NG group. The proportions greater than 50% in each group are shown for each biomarker

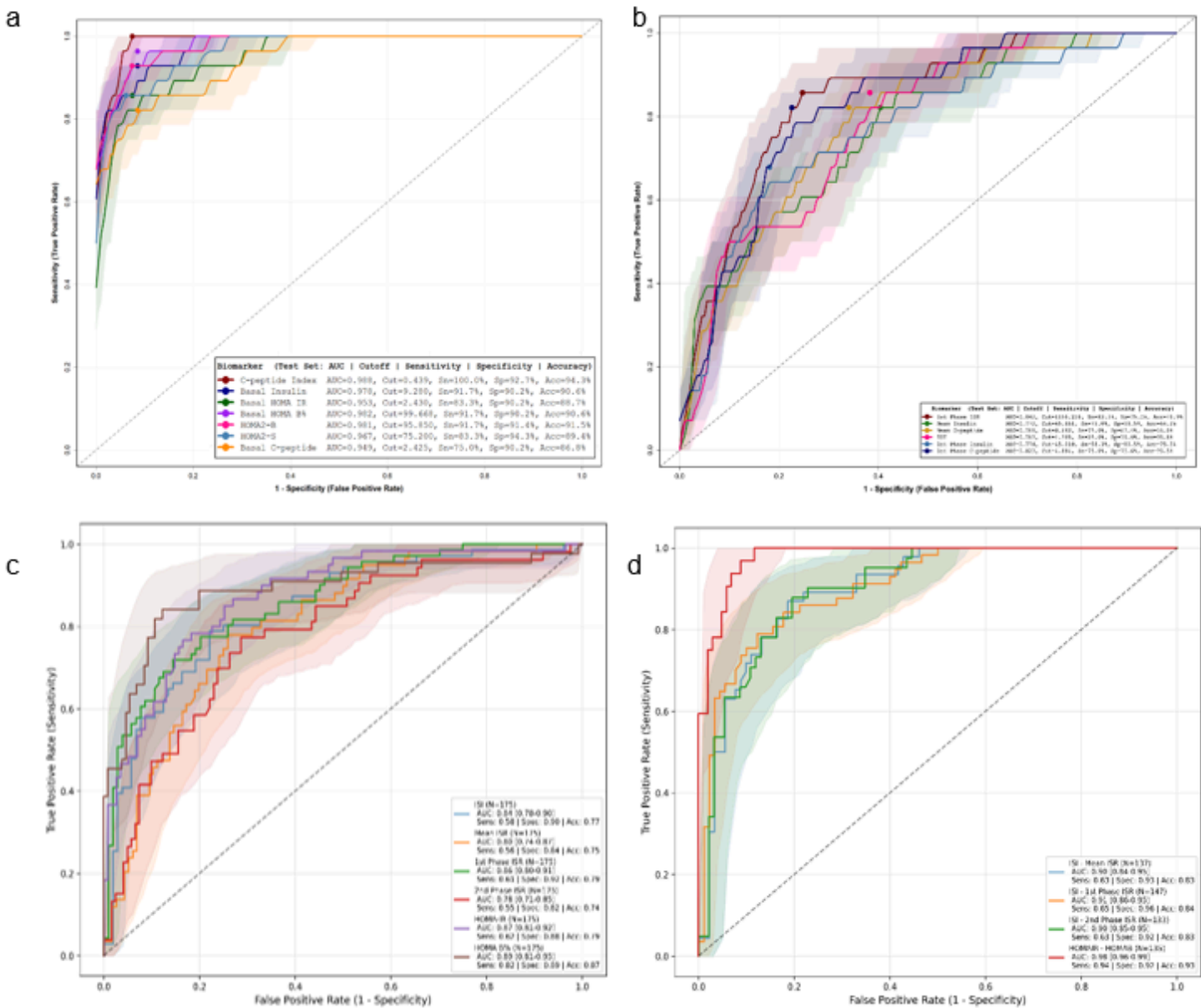


Figure 4

Diagnostic performance of basal and HG clamp measures

a) Bootstrap validation performance metrics for biomarker-based discrimination between ID and IR pre-diabetic subtypes. Performance was assessed using 1,000 bootstrap iterations with 70:30 train-test split (n=175; ID=135, IR=40). Values represent median test set performance with 95% confidence intervals in parentheses. Optimal cut-off values were determined using Youden's index. AUC, area under the receiver operating characteristic curve. Bootstrap validation of ROC curves for ID vs IR based on test set performance (1,000 iterations, 70:30 split). a) ROC performance for basal measures. Solid coloured lines

represent median test performance of the biomarkers. Shaded regions show 95% confidence intervals. Coloured dots mark optimal cut-offs from Youden's index. The dashed diagonal represents chance (AUC = 0.5). b) The ROC performance for HG clamp-based measures of 1st phase and mean insulin and C-peptide, ISR and ISI are shown. 1st phase C-peptide secretion provides a relatively better AUC of 0.842 in the secretion measures. c) The ROC curves for the diagnostic performance of c-peptide index cutoff =0.439 in discriminating low vs high HOMAB, HOMAIR, ISR and ISI measured by HG clamps. The curves are plotted for clamp based HOMAB, HOMAIR, ISI, 1st phase, 2nd phase and mean ISR. d) The ROC curves for the diagnostic performance of optimal c-peptide cut-off to discriminate IR and ID subtype stratified by high ISR-low ISI and low ISR-high ISI phenotypes based on HG clamp based ISI, 1st phase ISR and 2nd phase ISR, similarly it was assessed to classify low HOMAB-HOMAIR and high HOMAB-HOMAIR phenotype.

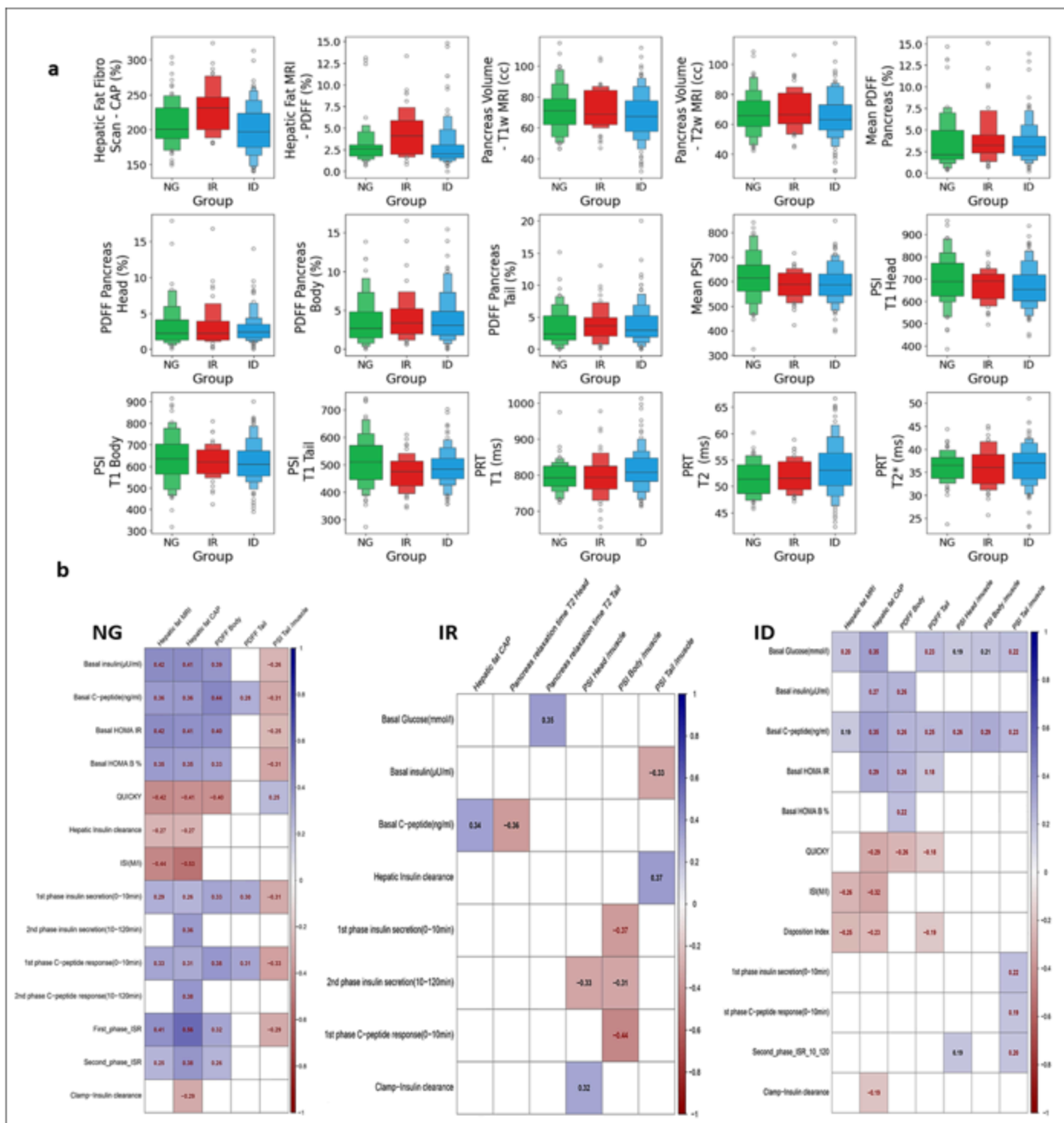


Figure 5

Hepatic and pancreatic imaging and the correlations with clamp-based indices

a) Comparison of hepatic and pancreatic imaging parameters among normal, IR and ID groups. Data are presented as median (IQR). Hepatic steatosis was assessed using FibroScan controlled attenuation parameter (CAP) and MRI PDFF (%). Pancreatic fat % (MRI PDFF), pancreatic volume, pancreas T1 signal intensity (PSI), and T1, T2, T2* relaxation times were assessed on multi parametric MRI and compared between the subgroups. Overall group differences were assessed using the ANOVA test, followed by

pairwise comparisons between the groups. b) the figure shows the statistically significant ($p < 0.05$) spearman correlational plots for basal and clamp variables with MRI measures for: Correlational plot for NG group, correlational plot for IR subtype, and correlational plot for ID subtype. The colour bar represents positive correlations by blue shade and negative correlations with red shade. The correlation coefficient that have $p < 0.05$ and $FDR < 0.1$ are highlighted in red"

Supplementary Files

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