Brief report

Lower hepatic fat is associated with improved insulin secretion in a high-risk prediabetes subphenotype during lifestyle intervention

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Keywords: prediabetes, cluster, lifestyle intervention, insulin secretion, insulin sensitivity, oral glucose tolerance test, hepatic triglyceride content, hepatic fat content, liver fat

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Figures: 1

Abstract

The objective of this work was to investigate whether impaired insulin secretion can be restored by lifestyle intervention in specific subphenotypes of prediabetes.

One thousand forty-five participants from the Prediabetes Lifestyle Intervention Study (PLIS) were assigned to 6 recently established prediabetes clusters. Insulin secretion was assessed by a C-peptide-based index derived from oral glucose tolerance tests and modeled from three time-points during a 1-yr intervention. We also analyzed the change of glycemia, insulin sensitivity and liver fat.

All pre-diabetes high-risk clusters (cluster 3, 5 and 6) had improved glycemic traits during lifestyle intervention, whereas insulin secretion only increased in clusters 3 and 5 ($p<0.001$); however, high liver fat in cluster 5 was associated with a failure to improve insulin secretion ($p_{interaction}<0.001$). Thus, interventions to reduce liver fat have the potential to improve insulin secretion in a defined subgroup of prediabetes.

Prediabetes is a heterogenous condition comprising subphenotypes with different risks of diabetes and its complications (1). From its two key features, insulin resistance and impaired insulin secretion, insulin resistance can be clearly improved by lifestyle intervention (LI); however, it is not known, if LI can improve insulin secretion in specific subphenotypes of reduced insulin secretion (2). Recently, we described 6 clusters of prediabetic metabolism (1). Two of these clusters (cluster 3 and 5) have high risk of progression to diabetes. Another group (cluster 6) has an intermediate risk of diabetes as these persons are capable of compensating insulin resistance via hyperinsulinemia over years. In this study, we retrospectively stratified participants of a large multi-center study into these novel clusters of prediabetic metabolism (1) and investigated whether LI improved their insulin secretion and other glycemic traits.

Research design and methods

Study population

The Prediabetes Lifestyle Intervention Study (PLIS) is a randomized controlled multi-center trial testing the efficacy of different intervention intensities in individuals with prediabetes (ClinicalTrials.gov identifier NCT01947595) (2). Participants with prediabetes were divided into a group with low-risk or a group with high-risk for diabetes progression. The low-risk group was then randomized to control or conventional LI, whereas the high-risk group was randomized to conventional or intensive LI for one year. While the control arm had only one 30-min consultation session with a dietitian, participants in the conventional and intensified arms received 8 or 16 recurring counselling sessions, respectively. The intention of counseling was to decrease body mass by 5% through reducing fat and increasing fiber intake. Participants with conventional and intensified intervention were also motivated to perform 3 or 6 hours of exercise per week, respectively. Postprandial glucose (glucose at 120 minutes after glucose challenge) was the primary endpoint of the study. Secondary endpoints were insulin sensitivity, liver fat and insulin secretion.

Assignment to metabolic clusters

Participants in the intention to treat analysis were assigned to metabolic clusters based upon several variables - comprising $AUC_{0\text{-}120}$glucose, insulin sensitivity, insulin secretion ($AUC_{0\text{-}30}$C-peptide/$AUC_{0\text{-}30}$glucose), HDL-cholesterol, visceral fat volume, subcutaneous fat volume, liver fat content and type 2 diabetes polygenic risk score, as described previously (1). Insulin and C-peptide were measured using the ADVIA Centaur XP Immunoassay System. Liver fat content was assessed with ^{1}H

magnetic resonance spectroscopy as described (2). Missing variables were imputed using multivariable imputation with chained equations (3) and complete cases set for the required variables was achieved for N=1045, see Suppl.Table1.

Outcome measures

Standardized 75g oral glucose tolerance tests (OGTTs) were performed at baseline, after 6 months and 12 months of LI. Insulin sensitivity was assessed by the Matsuda-index from 5-point insulin and glucose measurements (4). Results of the per-protocol analysis of the low-risk and high-risk groups of PLIS did not show an effect of LI on insulin secretion measured by an insulin-based index. We measured C-peptide as a post-hoc analysis of the study to assess insulin secretion by $AUC_{0\text{-}120}$C-peptide/$AUC_{0\text{-}120}$glucose, as this index had a lower coefficient of variation while still achieving high discrimination (5).

Statistics

Computations were performed with R (ver3.6.1). The change of outcome measures during lifestyle intervention was modelled with generalized linear mixed models applying the participant as random effect using the lme4 library. Fixed effects covariates comprised the intervention group, its interaction term with time, sex, age, BMI and time (since randomization). Insulin sensitivity was log-transformed when analyzed as outcome. For insulin secretion, further adjustment was performed for insulin sensitivity. To test how liver fat affects the change in insulin secretion, we fitted the interaction of time and liver fat, measured at the beginning and at the end of the trial, on insulin secretion in different prediabetes clusters. All tests were two-sided with an alpha level of 0.05. According to simulations performed with the simr

package(6), the statistical power to detect the change of insulin secretion in cluster 5 (ß=10, N_{groups}=213, $N_{measurements}$=594) was 76% (CI:66-84)%.

Data and Resource

Data of the PLIS study is currently not publicly available. Making the data publicly available without additional consent or ethical approval might compromise participant privacy and the original ethical approval. The R-code that supports this analysis is specific for the dataset of the PLIS study, and available upon request.

Results

All participants of the PLIS cohort met the criteria for prediabetes and ~82% (856 out of 1045) were assigned to the previously described high-risk clusters 3, 5 or 6. The baseline characteristics of participants stratified for metabolic clusters is shown in Suppl.Table 2. Due to the low number of participants assigned to the metabolically healthy obese cluster (cluster 4, N=8), this group was excluded from further analyses. There were no differences in renal function across the clusters that could impact assessment of insulin secretion through reduced C-peptide clearance (Suppl.Table 2). Before intervention, cluster 3 had the lowest insulin secretion independent from insulin sensitivity ($p<3.69*10^{-6}$, see Suppl. Table 2) and cluster 5 had the lowest insulin sensitivity compared to all other clusters (see Suppl.Table 3). We analyzed the change in key glycemic traits during LI incorporating all evaluation points (baseline, 6 months except for liver fat, and 12 months). Glycemia, insulin sensitivity, and liver fat content were improved by LI in all three high risk clusters (3, 5 and 6), independent from sex, age, BMI and the type of LI ($p<0.001$, see Figure 1.A-C). Insulin secretion improved independent from insulin sensitivity and the above mentioned covariates in cluster 3 and 5. However, participants in cluster 6, that is characterized by hyperinsulinemia, did not change their insulin secretion during LI (see Figure 1.D). Unadjusted insulin secretion also did not increase or decrease during LI ($p=0.4$).

We tested the hypothesis that the change of liver fat content—modeled as an interaction of time and MRS-derived hepatic fat content at study start and end—modulates insulin secretion using generalized linear mixed models with the fixed effect terms sex, age, BMI, insulin sensitivity, intervention, time × intervention and

time. There was a significant interaction between time and liver fat within cluster 5, but not the other tested clusters. The result suggests that lower liver fat during lifestyle intervention was associated with an increase of insulin secretion, whereas higher liver fat levels inhibited this improvement of insulin secretion (Figure 1.E). In contrast, with similarly constructed models there was no interaction with BMI in any tested cluster, suggesting that BMI does not impact the change of insulin secretion in either metabolic cluster (Suppl.Figure 1).

We also computed insulin secretion using the alternative index $AUC_{0\text{-}30}$C-peptide/$AUC_{0\text{-}30}$glucose that was used at the original cluster assignment. This index potentially involves hepatic insulin resistance (7) and yielded similar results (Suppl.Figure 2).

Conclusions

The data show that all previously defined high-risk clusters of prediabetes are amenable for improvement of glycemia, insulin sensitivity and liver fat content through LI; however, insulin secretion only improves in clusters 3 and 5. These two clusters are characterized by low insulin secretion for their respective insulin sensitivity. Of note, cluster 3 with only moderate insulin resistance and insulinopenia also improves insulin secretion. Cluster 6 did not increase insulin secretion during LI, which aligns with the observation that individuals in this cluster already have a prominent hyperinsulinemia. The lack of a decrease of insulin secretion (neither without nor with adjustment for insulin sensitivity) in cluster 6 suggests that hyperinsulinemia was not mitigated by the LI. Further studies are needed to

investigate the causes and therapeutic possibilities of hyperinsulinemia in this cluster.

Cluster 5 is characterized by insulin resistance, excessive liver fat content and inadequate insulin secretion (1). Our data suggest that liver fat content, but not body weight loss, is an important modulator of beta-cell function. Cell culture models show that a metabolic milieu characterized by fatty liver and insulin resistance promotes inflammatory cytokine production in adipose tissue adjacent to the pancreatic islets, which are in turn detrimental to insulin secretion(8). Therefore, lowering liver fat has the potential to relieve compromised beta-cell function in this prediabetes subphenotype.

Deterioration in β-cell function precedes the onset of type 2 diabetes(9), and increasing insulin secretion has been associated with lower diabetes risk in participants of the Diabetes Prevention Program(10). An improvement of beta-cell function paralleling liver fat reduction has been already shown in patients with diabetes(11), but has not yet been reported in prediabetes(12). Therapeutic strategies addressing hepatic fat reduction could be pivotal in improving insulin secretion and thereby preventing hyperglycemia in a subset of patients on a trajectory towards type 2 diabetes.

The assessment of a C-peptide-based insulin secretion index in this study allowed us to determine insulin secretion without interference from hepatic insulin clearance. However, even this OGTT-based index could inherently capture insulin resistance due to the physiologically intertwined nature of insulin secretion and insulin resistance. Also, we retrospectively analyzed data from an interventional study with different treatment arms, such that despite careful adjustment for treatment, a residual confounding might remain.

Our work identifies clusters of prediabetes patients who respond to LI with better beta cell function and delineates a group with particular benefits from liver fat reduction. Therapeutic modalities reducing liver fat content should be prospectively tested in future studies for high-risk individuals to prevent diabetes and its complications.

Article Information Section

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Author Contributions

R.W. contributed to data collection, researched data, and wrote the manuscript. M.H, K.K. J.M., F.S, L.F., A.P. contributed to data acquisition and discussion, reviewed and edited the manuscript. A.S., J.S., A.F.P., A.S, M.B., H.H., J.S., S.B., M.R., A.B., M.F.W contributed to discussion, edited and reviewed the manuscript. N.S., H.H. and A.F. designed the study, researched data, wrote and edited the manuscript.

Guarantor statement

R.W. and A.F. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Prior presentation

None

Duality of interest

RW reports lecture fees from NovoNordisk and Sanofi, and travel grants from Eli Lilly. He served on the advisory board of Akcea Therapeutics, Daiichi Sankyo, Sanofi and NovoNordisk. In addition to his current work, ALB reports lecture fees from Astra Zeneca, Boehringer Ingelheim, and NovoNordisk. He served on advisory boards of Astra Zeneca, Boehringer Ingelheim and NovoNordisk. Besides his current work, AF reports lecture fees and advisory board membership from Sanofi, Novo Nordisk, Eli Lilly, and AstraZeneca. In addition to his current work, MH reports research grants from Boehringer Ingelheim and Sanofi (both to the University Hospital of Tuebingen) and lecture fees from Sanofi, Novo Nordisk, Eli Lilly and Merck Sharp Dohme. MFW is a scientific consultant for Housey Pharmaceutical Research Laboratories. None of the other authors report a conflict of interest directly related to the content of this work.

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Figures

Figure 1.A-E

Cluster-specific change of glycemia (A), insulin sensitivity (B), liver fat (C) and insulin secretion (D) during lifestyle intervention with influence of liver fat on change of insulin secretion during lifestyle-intervention (E). Traits are shown as residuals from generalized linear mixed models adjusted for sex, age, BMI, time x intervention (control, conventional or intensive), and in the case of insulin secretion additionally for insulin sensitivity as fixed effects. Effect sizes (ß) and p-values are provided for the term time. Cluster-wise interactions between hepatic fat content and time in generalized linear mixed models are shown as marginal effects by plotting the modelled change of insulin secretion for low (mean-SD), mid (mean) and high (mean+SD) hepatic fat content. Effect sizes (ß) and p-values are provided for the interaction between liver fat and time.

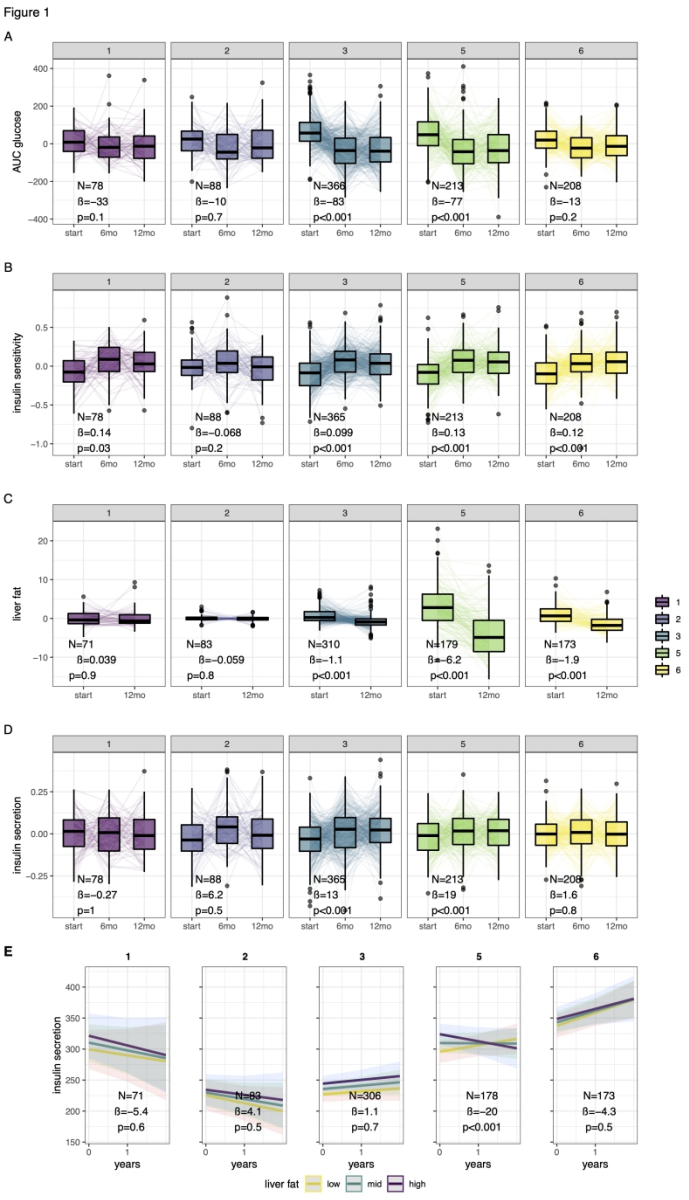


Figure 1. A-E Cluster-specific change of glycemia (A), insulin sensitivity (B), liver fat (C) and insulin secretion (D) during lifestyle intervention with influence of liver fat on change of insulin secretion during lifestyle-intervention (E). Traits are shown as residuals from generalized linear mixed models adjusted for sex, age, BMI, time x intervention (control, conventional or intensive), and in the case of insulin secretion additionally for insulin sensitivity as fixed effects. Effect sizes (ß) and p-values are provided for the term time. Cluster-wise interactions between hepatic fat content and time in generalized linear mixed models are shown as marginal effects by plotting the modelled change of insulin secretion for low (mean-SD), mid (mean) and high (mean+SD) hepatic fat content. Effect sizes (ß) and p-values are provided for the interaction between liver fat and time.

1058x1799mm (72 x 72 DPI)

Supplementary Data

Supplementary Tables

Supplementary Table 1

Cross-imputation of missing variables (N=1045)

Variable	Missing rate
MRI subcutaneous fat volume	0.39
MRI total fat volume	0.39
MRI visceral fat volume	0.39
MR spectroscopy liver fat	0.17
Fat percentage (bioimpedance)	0.05
Fasting insulin	0.02
Post-challenge insulin 120 min	0.02
Insulin sensitivity (Matsuda-index)	0.02
Insulin secretion (C-peptide based)	0.02
Glycemia (AUC glucose)	0.01
Hip circumference	0.01
Waist circumference	0.01
BMI	0
Fasting glucose	0
Post-challenge glucose 120 min	0
Alanin-aminotransferase	0
Sex	0
Triglycerides	0

Supplementary Table 2

Participants of the Prediabetes Lifestyle Intervention Study (PLIS) at baseline stratified to previously established metabolic clusters. Differences were tested with ANOVA.

Cluster	1	2	3	4	5	6	p-value
Metabolic risk*	Low	Very Low	High	Low	High	High	
Key features*			Low insulin secretion without pronounced insulin resistance	Metabolically healthy obesity	Prominent insulin resistance with insulin secretion failure	Insulin resistance with compensatory hyperinsulinemia	
n	88	93	399	8	229	228	
Intervention group (%)							<0.001
conventional	43 (48.9)	40 (43.0)	194 (48.6)	6 (75.0)	111 (48.5)	128 (56.1)	
control	36 (40.9)	29 (31.2)	30 (7.5)	1 (12.5)	0 (0.0)	20 (8.8)	
intensive	9 (10.2)	24 (25.8)	175 (43.9)	1 (12.5)	118 (51.5)	80 (35.1)	
Sex = male (%)	49 (55.7)	45 (48.4)	183 (45.9)	2 (25.0)	77 (33.6)	81 (35.5)	<0.001
Age (mean (SD))	57.37 (11.78)	59.52 (11.38)	60.17 (9.80)	51.30 (11.23)	56.42 (9.55)	56.62 (11.09)	<0.001
BMI (kg/m2) (mean (SD))	27.59 (4.84)	24.62 (2.68)	29.07 (4.43)	32.69 (3.60)	34.45 (5.57)	35.28 (5.48)	<0.001
eGFR (ml/min/1.73m2) (mean (SD))	90.88 (15.53)	90.72 (13.36)	89.33 (12.99)	102.33 (12.24)	91.22 (13.86)	91.20 (15.37)	0.082
AUC glucose (mean (SD))	894.20 (93.36)	936.37 (138.93)	1158.91 (143.98)	824.27 (71.25)	1198.05 (154.10)	1006.56 (134.60)	<0.001
Insulin secretion ($AUC_{0\text{-}120}$ C-peptide/$AUC_{0\text{-}120}$ glucose) **(AU) (mean (SD))**	309.09 (79.65)	209.59 (58.59)	229.21 (60.43)	241.60 (46.43)	293.29 (76.21)	343.06 (80.92)	<0.001
insulin sensitivity (AU) (mean (SD))	7.97 (3.34)	13.78 (5.16)	7.09 (3.01)	10.15 (2.49)	3.83 (1.85)	4.59 (1.93)	<0.001
HDL_[mmol/l] (mean (SD))	1.34 (0.32)	1.98 (0.76)	1.45 (0.36)	1.35 (0.24)	1.25 (0.28)	1.31 (0.33)	<0.001
subcutaneous fat volume (l) (mean (SD))	9.58 (3.19)	8.23 (3.94)	12.41 (4.73)	18.36 (7.31)	17.69 (6.79)	20.49 (13.27)	<0.001
visceral fat volume (l) (mean (SD))	3.89 (1.58)	2.84 (1.53)	4.92 (1.97)	4.94 (1.51)	6.14 (2.17)	6.96 (3.75)	<0.001
liver fat content (%) (mean (SD))	3.23 (2.91)	2.28 (2.59)	5.47 (4.10)	3.67 (2.63)	20.62 (6.94)	8.81 (4.58)	<0.001
polygenic risk score (mean (SD))	-0.31 (0.95)	0.00 (0.93)	0.24 (1.01)	-0.56 (1.03)	0.02 (0.92)	-0.10 (1.00)	<0.001

*see Ref. 1 (Wagner et al, Nature Medicine 2021)

Supplementary Table 3

Between-cluster comparison of key anthropometric and metabolic measures (two-sided ANOVA with post-hoc test using Tukey's method)

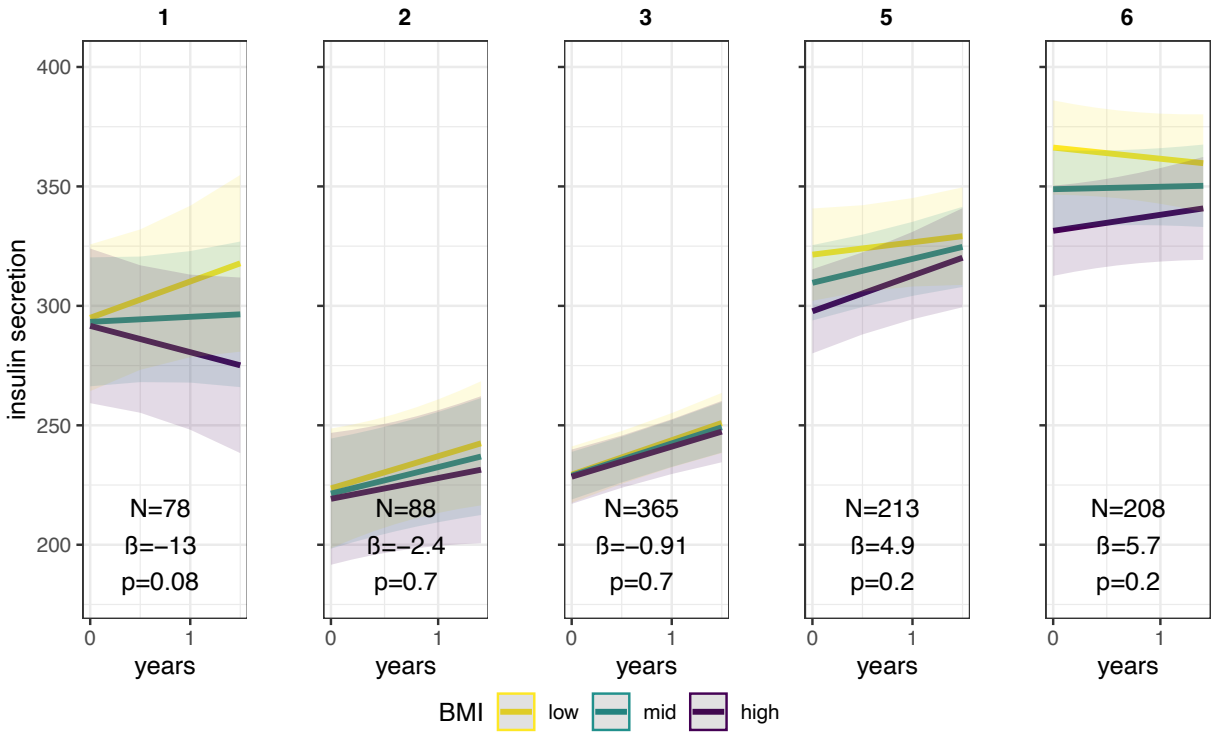
	2-1	3-1	3-2	5-1	5-2	5-3	6-1	6-2	6-3	6-5
Age	0.63	0.15	0.98	0.95	0.11	$1.33*10^{-04}$	0.98	0.16	$3.82*10^{-04}$	1
BMI (kg/m2)	$4.06*10^{-04}$	0.074	$4.97*10^{-14}$	$<1*10^{-14}$	$<1*10^{-14}$	$<1*10^{-14}$	$<1*10^{-14}$	$<1*10^{-14}$	$<1*10^{-14}$	0.36
AUC glucose	0.26	$<1*10^{-14}$	$<1*10^{-14}$	$<1*10^{-14}$	$<1*10^{-14}$	0.0071	$2.64*10^{-09}$	$4.92*10^{-04}$	$<1*10^{-14}$	$<1*10^{-14}$
Insulin secretion (AU)	$1.49*10^{-13}$	$1.48*10^{-13}$	0.12	0.39	$1.47*10^{-13}$	$1.38*10^{-13}$	0.0014	$1.01*10^{-13}$	$1.01*10^{-13}$	$1.77*10^{-12}$
Insulin secretion adjusted (AU)*	0.0092	$3.28*10^{-13}$	$4.09*10^{-13}$	$4.32*10^{-13}$	$3.83*10^{-04}$	$3.69*10^{-06}$	0.99	0.0045	$3.28*10^{-13}$	$3.76*10^{-13}$
insulin sensitivity (AU)	$6.22*10^{-14}$	0.08	$6.22*10^{-14}$	$7.46*10^{-14}$	$6.22*10^{-14}$	$6.22*10^{-14}$	$1.12*10^{-13}$	$6.22*10^{-14}$	$1.10*10^{-13}$	0.044
HDL (mmol/l)	$<1*10^{-14}$	0.092	$<1*10^{-14}$	0.4	$<1*10^{-14}$	$8.36*10^{-09}$	0.98	$<1*10^{-14}$	$1.04*10^{-04}$	0.52
subcutaneous fat volume (l)	0.84	0.059	$4.10*10^{-04}$	$<1*10^{-14}$	$<1*10^{-14}$	$2.78*10^{-10}$	$<1*10^{-14}$	$<1*10^{-14}$	$<1*10^{-14}$	0.019
visceral fat volume (l)	0.093	0.022	$3.54*10^{-09}$	$1.43*10^{-08}$	$<1*10^{-14}$	$1.61*10^{-05}$	$<1*10^{-14}$	$<1*10^{-14}$	$<1*10^{-14}$	0.045
liver fat content (%)	0.71	0.0021	$4.17*10^{-07}$	$<1*10^{-14}$	$<1*10^{-14}$	$<1*10^{-14}$	$<1*10^{-14}$	$<1*10^{-14}$	$7.06*10^{-13}$	$<1*10^{-14}$
polygenic risk score	0.19	$2.23*10^{-05}$	0.24	0.051	1	0.065	0.4	0.92	$4.33*10^{-04}$	0.69

*adjusted for insulin sensitivity

Supplementary Figures

Supplementary Figure 1

Cluster-wise interactions between BMI and time in generalized linear mixed models are shown as marginal effects by plotting the modelled change of insulin secretion for low (mean-SD), mid (mean) and high (mean+SD) BMI. Effect sizes (ß) and p-values are provided for the interaction between BMI and time.



Supplementary Figure 2. A-B

Sensitivity analysis investigating cluster-specific change of insulin secretion (A) and time x liver fat interaction (B) using **$AUC_{0\text{-}30}$ C-peptide/$AUC_{0\text{-}30}$ glucose** as insulin secretion index (outcome). Otherwise, these models feature the same exposure variables as the models shown in Figure 1. A, D