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Clinical Research Article

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A Pathophysiology of Type 2 Diabetes Unrelated to Metabolic Syndrome

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Abstract

Objective: Clinically, type 2 diabetes mellitus (T2DM) is heterogeneous, but the prevailing pathophysiologic hypothesis nevertheless contends that components of metabolic syndrome are central to all cases of T2DM. Here, we re-evaluated this hypothesis.

Research Design and Methods: We conducted a cross-sectional analysis of 138 women from the monocenter, post gestational diabetes study PPSDiab, 73 of which had incident prediabetes or T2DM. Additionally, we examined all the 412 incident cases of T2DM in phases 3 to 9 of the Whitehall II study in comparison to healthy controls. Our analysis included a medical history, anthropometrics, oral glucose tolerance testing, and laboratory chemistry in both studies. Additional analyses from the PPSDiab Study consisted of cardiopulmonary exercise testing, magnetic resonance imaging, autoantibody testing, and the exclusion of glucokinase maturity-onset diabetes of the young.

Results: We found that 33 (45%) of the women with prediabetes or T2DM in the PPSDiab study displayed no components of metabolic syndrome. They reached no point for metabolic syndrome in the National Cholesterol Education Program Adult Treatment Panel III score other than hyperglycemia and, moreover, had levels of liver fat content, plasma triglycerides, high-density lipoprotein cholesterol, c-reactive protein, and blood pressure that were comparable to healthy controls. In the Whitehall II study, 62 (15%) of the incident T2DM cases fulfilled the same criteria. In both studies, these cases without metabolic syndrome revealed insulin resistance and inadequately low insulin secretion.

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Conclusions: Our results contradict the hypothesis that components of metabolic syndrome are central to all cases ofT2DM. Instead, they suggest the common occurrence of a second, unrelated pathophysiology.

Key Words: type 2 diabetes, subtypes, subclassifications, metabolic syndrome, insulin resistance, insulin secretion

Although obesity remains a major risk factor for type 2 diabetes mellitus (T2DM), the disease can also affect lean individuals (1). Furthermore, the relative contribution of insulin resistance *vs* impaired insulin secretion varies between individuals, as does the course of the disease over time (2). Despite this obvious clinical heterogeneity, pathophysiologic research still focuses on the one hypothesis that components of metabolic syndrome, in particular ectopic lipid deposition and low-grade inflammation, are central to all cases of T2DM. In lean individuals, a metabolically unhealthy phenotype is proposed, which displays components of metabolic syndrome already starting at a low "personal fat threshold" (1).

We tested this hypothesis of a single, metabolic syndrome-related pathophysiology of T2DM in 2 complementary human studies. Initially, we analyzed the monocenter, prospective, deep-phenotyping Prediction, Prevention and Subclassification of Type 2 Diabetes (PPSDiab) study. This study focused on young women with a recent history of gestational diabetes mellitus (GDM) and on a control group of women who had a normoglycemic pregnancy. Because GDM is a strong risk marker for T2DM (3), this design permitted the detailed examination of cases in early, mainly prediabetic stages of T2DM development. To validate our findings, we then analyzed data from the Whitehall II study, a large, prospective cohort study. In this data set, we characterized all cases with incident T2DM.

We applied a 2-step approach in both studies. In the first step, we selected the fraction of the cases not reaching any point for metabolic syndrome according to the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP3) score (2)—with the exception of hyperglycemia, which we treated separately because, naturally, it was part of the case definition. In the second step, we examined the selected fraction of the cases for other components of metabolic syndrome not reflected in the clinical score. In this step, we focused on comparisons to matched healthy control subjects.

Materials and Methods

PPSDiab study

Study design and cohort

Women included in our analysis were participants of the ongoing, prospective, monocenter observational PPSDiab study, enrolled between November 2011 and May 2016. The study has already been described in detail elsewhere (4,5). Briefly, the cohort of the PPSDiab study consists of women who suffered from GDM during their last pregnancy and of women following a normoglycemic pregnancy in a ratio of 2:1. In total, 304 women were recruited consecutively from the Diabetes Center and the Obstetrics Department of the university hospital of the Ludwig-Maximilians-Universität in Munich, Germany. Exclusion criteria for this study included alcohol or substance abuse, prepregnancy diabetes, and chronic diseases requiring systemic medication [except for hypothyroidism (n = 52), mild hypertension (n = 4), gastroesophageal reflux (n = 2), and a history of pulmonary embolism resulting in rivaroxaban prophylaxis (n = 1)]. Written informed consent was obtained from all study participants, and the study protocol was approved by the ethical review committee of the Ludwig-Maximilians-Universität in Munich, Germany.

Medical history and questionnaires

A detailed medical history was obtained and the participants answered questionnaires concerning their physical activity, eating habits, psychosocial situation, and well-being.

Anthropometrics and body composition

Systolic and diastolic blood pressure readings were obtained in a sitting position with repeated measurements on both arms from which the average from the "higher" arm was recorded. Body weight and fat mass were measured using a bioelectrical impedance analysis scale (Tanita BC-418, Tanita Corporation). Waist circumference and height were measured to the nearest centimeter.

Oral and intravenous glucose tolerance test

A 5-point, 75-g oral glucose tolerance test (oGTT) was conducted with measurements of plasma glucose and serum insulin. An intravenous glucose tolerance test was performed in all study participants available for a second day of testing. For the test, a glucose bolus of 0.3 g/kg body weight was injected over one minute. Blood samples were drawn at 0, 2, 4, 6, 8, 10, 20, 30, 45, and 60 minutes. Several metabolic indices were calculated from these tests, which are detailed in Table 1.

Clinical chemistry and auto-antibody testing

Plasma glucose was measured with the Glucose HK Gen.3 assay (Roche Diagnostics), serum triglycerides and

high-density lipoprotein (HDL) cholesterol was determined by enzymatic caloric tests (Roche Diagnostics), high-sensitivity c-reactive protein was recorded by Wide-Range CRP (Siemens Healthcare Diagnostics), nonesterified fatty acids (NEFA) were quantified by a calorimetric assay (Wako Chemicals), and insulin and C-peptide by a LIAISON chemiluminescence immunoassay (DiaSorin). For the measurement of autoantibodies, we used a radioimmunoassay (CentAK anti-GAD65 Monoclonal; article number 2071; Medipan; cutoff 2,0 U/mL) for glutamic acid decarboxylase 65-kilodalton isoform, a radioimmunoassay (CentAK anti-IA2M; article number 2050; Medipan; cutoff 2,0 U/mL) for islet antigen 2, and an enzyme-linked immunosorbent assay for Zink Transport-Factor 8 (ZnTF8) (Anti-Zink-Transporter-8-ELISA; article number 1027–9601; Euroimmun; cutoff 15 U/mL).

Cardiopulmonary exercise testing

Cardiopulmonary exercise testing was performed on a bicycle ergometer using the spiroergometry system MasterScreen CPX (Care Fusion). The ergometry protocol consisted of stepwise increments of 25 watts every 3 minutes and started with a reference phase without load. Electrocardiogram, oxygen uptake, and carbon dioxide exhalation were recorded continuously and participants were asked to rate their perceived exertion by pointing to a BORG scale (10). The test was terminated when the participant was exhausted and maximum achieved oxygen uptake (VO_2peak) was recorded. A maximal respiratory exchange ratio of at least 1.05 was required for a valid exercise test.

Magnetic resonance imaging and magnetic resonance

Whole-body magnetic resonance imaging examinations were performed with a 3 Tesla system (Ingenia or Achieva, Philips Healthcare) in all participants available for a third day of testing. We measured whole-body fat and intra-abdominal adipose tissue volume. Liver fat estimates were derived from a modified 2-point Dixon sequence (4).

A detailed description of the study's standard operating procedures, anthropometric and clinical measurements as well as methodologies of blood sampling and analysis can be found elsewhere (4,5).

Exclusion of glucokinase–maturity-onset diabetes of the young

Whole blood genomic DNA was isolated from all women with prediabetes/T2DM but 0 additional points for metabolic syndrome using a standard protocol (11). Coding exons and flanking intronic sequences of the *GCK* gene [glucokinase (GCK]) were amplified by standard polymerase chain reaction using in-house validated primers. Distinct polymerase chain reaction products were verified by agarose gel electrophoresis and subsequently purified by Agencourt AMPure XP System on a Biomek NXP Automated Workstation (Beckman Coulter). Purified amplicons were sequenced bidirectionally using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) on 3730xl DNA Analyzer (Applied Biosystems). Sequences were analyzed and compared with the published reference sequence (ENSEMBL ENST00000403799) using SeqPilot.

Table 1. Metabolic indices calculated in the PPSDiab study

Index	Formula
Insulin sensitivity index (6)	10 000 / (SQRT [PG0' * INS0' * (PG0' * 15 + PG30' * 30 + PG60' * 30 + PG90' * 30 + PG120' * 15) / 120 * (INS0' * 15 + INS30' * 30 + INS60' * 30 + INS90' * 30 + INS120' * 15) / 120])
Δins 30' (4)	INS30" – INS0"
Disposition index (7)	Δins 30' * ISI
ΔC-Pept. 30'	C-Pept.30' – C-Pept.0'
Adaptation index (8)	ΔC-Pept. 30' * ISI
Hepatic insulin resistance index (9)	AUC PG(0'-30') * AUC INS(0"-30") / 10 000
Muscle insulin sensitivity index (9)	dPG/dt(peak to nadir) / mean INS
First-phase insulin secretion (4)	AUC INS(0'-10') / INS0' * 10
Intravenous Disposition Index (ivDI) (4)	FPIR * ISI

Abbreviations: AUC, area under the curve; C-Pept., C-peptide (ng/mL); INS, serum insulin (µU/mL); PG, plasma glucose (mg/dL); SQRT, square root.

Whitehall II study

Study design and cohort

For the Whitehall II study, a cohort of 10 308 British civil servants aged 35 to 55 was recruited in 1985 and has since been followed up in several phases in 2- to 5-year intervals (12). Here, we used data from study phase 3 (1991-1994), phase 5 (1997-1999), phase 7 (2002-2004), and phase 9 (2007-2009) for our analysis. These phases include all in-person visits with glucose tolerance testing from 1991-2009. The Whitehall II study was reviewed and approved by the University College London Ethics Committee. All participants gave written informed consent at each study phase.

Data collection

At each study phase, a medical history and questionnaires were obtained. Additionally, anthropometric and laboratory chemistry parameters were measured. A 75-g oGTT

with measurements of fasting and 2-h plasma glucose as well as fasting and 2-h serum insulin was offered to all participants. The metabolic indices calculated from this test are detailed in Table 2.

Definitions of prediabetes, diabetes, and metabolic syndrome in both studies

In both studies, prediabetes and diabetes were defined according to the American Diabetes Association (13). In the PPSDiab study, the oGTT at the study visit was the only criterium for incident prediabetes or diabetes. In the Whitehall II study, we defined all cases with a first diagnosis of diabetes determined by the study oGTT or who reported a new diagnosis made elsewhere at the study visit as incident diabetes.

For metabolic syndrome, we applied the NCEP ATP3 score (14) that uses waist circumference, triglycerides, HDL cholesterol, blood pressure, and fasting plasma glucose/a diagnosis of diabetes to assign up to 5 points for metabolic syndrome. The cutoff for a clinical diagnosis of metabolic syndrome is 3 or more points.

In the case groups of both studies, we ignored the point for disturbed glucose metabolism for our analysis, as this was part of the case definition. Thus, we grouped the cases according to the 4 non-hyperglycemia points.

Groups Pre0 and Diab0, respectively, had 0 non-hyperglycemia points and thus displayed a waist circumference <88 cm in women and <102 cm in men and HDL cholesterol ≥ 50 mg/dL in women and ≥ 40 mg/dL in men without medication, and triglycerides < 150 mg/dL without medication, and blood pressure < 130/85 mmHg without medication.

Groups Pre≥1 and Diab≥1, respectively, had ≥1 non-hyperglycemia points and thus did not meet 1 or more of the previously described criteria.

The healthy control groups in both studies displayed 0 out of the conventional 5 points of the NCEP ATP3 score.

Statistical analysis

All metric and normally distributed variables are reported as mean ± SD. Nonnormally distributed variables are reported as median (first quartile-third quartile) and categorical variables as frequency and percentage. Group comparisons were performed based on the Kruskal-Wallis, Mann-Whitney-U, or signed Wilcoxon rank test for metric variables and the chi-square or Fisher's exact test for categorical variables. For the Kruskal-Wallis post-hoc analysis, Dunn's procedure was applied. To compare the discriminatory power of different metabolic indices, binary logistic regression models were calculated. *P*-values < 0.05 were considered statistically significant. All statistical calculations were performed using SAS statistical software package version 9.4 (SAS Institute, Inc., Cary, NC, USA) or R version 3.5.1 (http://www.R-project.org).

Table 2. Metabolic indices calculated in the Whitehall II study

Index	Formula
Insulin sensitivity index from 2 time points	10 000 / SQRT (PG0' * INS0' * PG120' * INS120')
Matsuda and DeFronzo (ISI) (16)	
HOMA-1B (17)	(20 × fasting plasma insulin) / (fasting plasma glucose – 3.5)

Abbreviations: INS, serum insulin (μU/mL); PG, plasma glucose (mg/dL for ISI; mmol/L for HOMA-1B); SQRT, square root.

Results

PPSDiab study

First, we performed a cross-sectional analysis of the PPSDiab study using data from the study's baseline visit (Table 3; Fig. 1). Here, we compared 3 groups of women. The first group, the Control group (n = 65), was defined as women who were normoglycemic at the study visit, had previously experienced a normoglycemic pregnancy, and had 0 points for metabolic syndrome. The second group, the Pre0 case group (n = 33; 45% of all cases), included women who had prediabetes (n = 30) or T2DM (n = 3) at the study visit, had 0 non-hyperglycemia points for metabolic syndrome, and previously had experienced GDM. The third group, the Pre≥1 case group (n = 40; 55% of all cases), was equivalent to Pre0, except for the presence of at least 1 non-hyperglycemia point for metabolic syndrome. In Pre≥1, 34 women had prediabetes and 6 had T2DM. All women from the PPSDiab study cohort, who fitted one of the group definitions, were included in this analysis.

As a result of the group definitions used, fasting and 2-h plasma glucose were elevated in Pre0 and Pre≥1 compared to Control. However, both values were not significantly different between Pre0 and Pre≥1. Likewise, due to the group definitions, Pre≥1 exhibited signs of obesity and metabolic syndrome in comparison to Control and Pre0 [ie, higher body mass index (BMI), waist circumference, body fat content, systolic and diastolic blood pressure, triglycerides, c-reactive protein, abdominal visceral fat, and liver fat content as well as lower HDL cholesterol. However, none of these parameters differed significantly between Control and Pre0 (Table 3; Fig. 1).

Table 3. Characteristics of Control, Pre0, and Pre≥1 in the PPSDiab study

	Control	Pre0	Pre≥1	*P*-value[a]	Significant post hoc tests[b]
n	65	33	40		
Age, years	35.5 ± 4.1	36.2 ± 3.9	35.7 ± 5.2	0.70	–
Time since delivery, months	9.5 ± 2.6	10.2 ± 3.2	8.8 ± 3.0	0.21	–
Glucose tolerance status					
NGT	65 (100%)	—	—	<0.0001	–
IFG		19 (57.6%)	12 (30.0%)		
IGT		9 (27.3%)	14 (35.0%)		
IFG and IGT		4 (12.1%)	8 (20.0%)		
Diabetes		1 (3.0%)	6 (15.0%)		
Active smoker					–
Yes	4 (6.2%)	0 (0%)	8 (20%)	0.007	
No	61 (93.8%)	33 (100%)	32 (80%)		
Hormonal contraception					
No	53 (81.5%)	27 (81.8%)	29 (72.5%)	0.10	–
Gestagene	10 (15.4%)	5 (15.2%)	4 (10.0%)		
Combination	2 (3.1%)	1 (3.0%)	7 (17.5%)		
Systolic BP, mmHg	110.6 ± 8.0	113.2 ± 8.1	127.1 ± 12.7	<0.0001	Pre≥1 *vs* Control, Pre≥1 *vs* Pre0
Diastolic BP, mmHg	68.1 ± 6.8	70.0 ± 6.3	80.2 ± 10.0	<0.0001	Pre≥1 *vs* Control, Pre≥1 *vs* Pre0
BMI, kg/m^2	22.3 ± 2.4	22.8 ± 2.8	32.5 ± 6.3	<0.0001	Pre≥1 *vs* Control, Pre≥1 *vs* Pre0
Waist circumference, cm	74.4 ± 5.4	76.6 ± 6.5	94.6 ± 11.2	<0.0001	Pre≥1 *vs* Control, Pre≥1 *vs* Pre0
Body fat, %]	27.9 ± 5.8	29.2 ± 6.9	40.5 ± 6.7	<0.0001	Pre≥1 *vs* Control, Pre≥1 *vs* Pre0
Liver fat, % (n = 79)	0.1 (0.0-0.6)	0.1 (0.0-0.9)	7.3 (3.2-12.1)	<0.0001	Pre≥1 *vs* Control, Pre≥1 *vs* Pre0
Intra-abdominal fat	1.2 (0.8-1.6)	1.4 (1.0-2.4)	2.8 (2.0-3.8)	<0.0001	Pre≥1 *vs* Control, Pre≥1 *vs* Pre0
V0$_2$peak, mL/min (n = 112)	1926 (1744-2158)	1686 (1397-1846)	1767 (1604-1961)	0.0010	Pre0 *vs* Control, Pre≥1 *vs* Control
hsCRP, mg/dL	0.02 (0.01-0.05)	0.03 (0.01-0.06)	0.18 (0.07-0.47)	<0.0001	Pre≥1 *vs* Control, Pre≥1 *vs* Pre0
Triglycerides, mg/dL	59.0 (50.0-72.0)	68.0 (57.0-77.0)	111.0 (79.5-172.0)	<0.0001	Pre≥1 *vs* Control, Pre≥1 *vs* Pre0
HDL cholesterol, mg/dL	64.0 (57.0-76.0)	59.0 (53.0-71.0)	49.5 (42.5-60.0)	<0.0001	Pre≥1 *vs* Control, Pre≥1 *vs* Pre0
NEFA, µmol/L	555 (379-739)	675 (587-780)	628 (542-737)	0.024	Pre0 *vs* Control
Fasting PG, mmol/L	4.9 (4.6-5.1)	5.7 (5.4-5.8)	5.7 (5.4-5.9)	<0.0001	Pre0 *vs* Control, Pre≥1 *vs* Control
2-h PG, mmol/L	4.9 (4.4-5.9)	6.8 (5.8-8.3)	8.3 (7.1-10.1)	<0.0001	Pre0 *vs* Control, Pre≥1 *vs* Control
Fasting insulin, µU/mL	5.3 (4.0-6.6)	8.1 (6.3-12.9)	12.6 (9.0-18.0)	<0.0001	Pre0 *vs* Control, Pre≥1 *vs* Control
ISI	7.6 (5.7-9.3)	4.1 (3.0-5.1)	2.6 (2.0-3.6)	<0.0001	all
$^{\Delta}$ins 30'	37.5 (29.3-53.8)	36.5 (29.4-67.9)	57.8 (35.4-82.0)	0.015	Pre≥1 *vs* Control
DI	302 (241-363)	153 (131-204)	158 (100-205)	<0.0001	Pre0 *vs* Control, Pre≥1 *vs* Control
$^{\Delta}$C-peptide 30', ng/mL	3.9 (3.2-4.9)	4.0 (3.5-5.7)	4.9 (3.5-6.4)	0.0454	Pre≥1 *vs* Control
Beta Cell Adaptation Index	30.0 (21.7-35.5)	17.2 (11.5-21.7)	11.3 (8.8-18.8)	<0.0001	Pre0 *vs* Control, Pre≥1 *vs* Control
Hepatic Insulin Resistance Index	238.3 (189.8- 355.0)	352.1 (293.4-512.8)	502.4 (347.3-750.3)	<0.0001	Pre0 *vs* Control, Pre≥1 *vs* Control
Muscle Insulin Sensitivity Index (n = 119)	16.9 (11.9-26.1)	13.3 (9.1-17.9)	7.3 (4.2-11.2)	<0.0001	Pre0 *vs* Control, Pre≥1 *vs* Control
FPIR (n = 76)	2.0 (1.3-2.5)	1.9 (1.1-2.5)	3.4 (2.1-4.0)	0.039	Pre≥1 *vs* Control, Pre≥1 *vs* Pre0
ivDI (n = 76)	15.5 (10.4-20.2)	6.9 (4.9-9.8)	7.3 (5.1-11.7)	<0.0001	Pre0 *vs* Control, Pre≥1 *vs* Control

For details on metabolic indices see Table 1.

Abbreviations: BMI, body mass index; BP, blood pressure; $^{\Delta}$ins 30', rise of serum insulin during the first 30 minutes of the oral glucose tolerance test (oGTT); DI, disposition index calculated from oGTT; FPIR, first-phase insulin release in the intravenous glucose tolerance test; hsCRP, high-sensitivity c-reactive protein; ISI, insulin sensitivity index; ivDI, disposition index calculated from FPIR and ISI; NEFA, nonesterified fatty acids; oGTT PG, plasma glucose; VO$_2$peak, peak oxygen uptake in cardiopulmonary exercise testing.

[a]*P*-value of Kruskal-Wallis test for metric and of chi-square or Fisher's exact test for categorical variables.

[b]For metric variables with a *P*-value <0.05, the post-hoc Dunn's procedure was applied to determine pairwise significance.

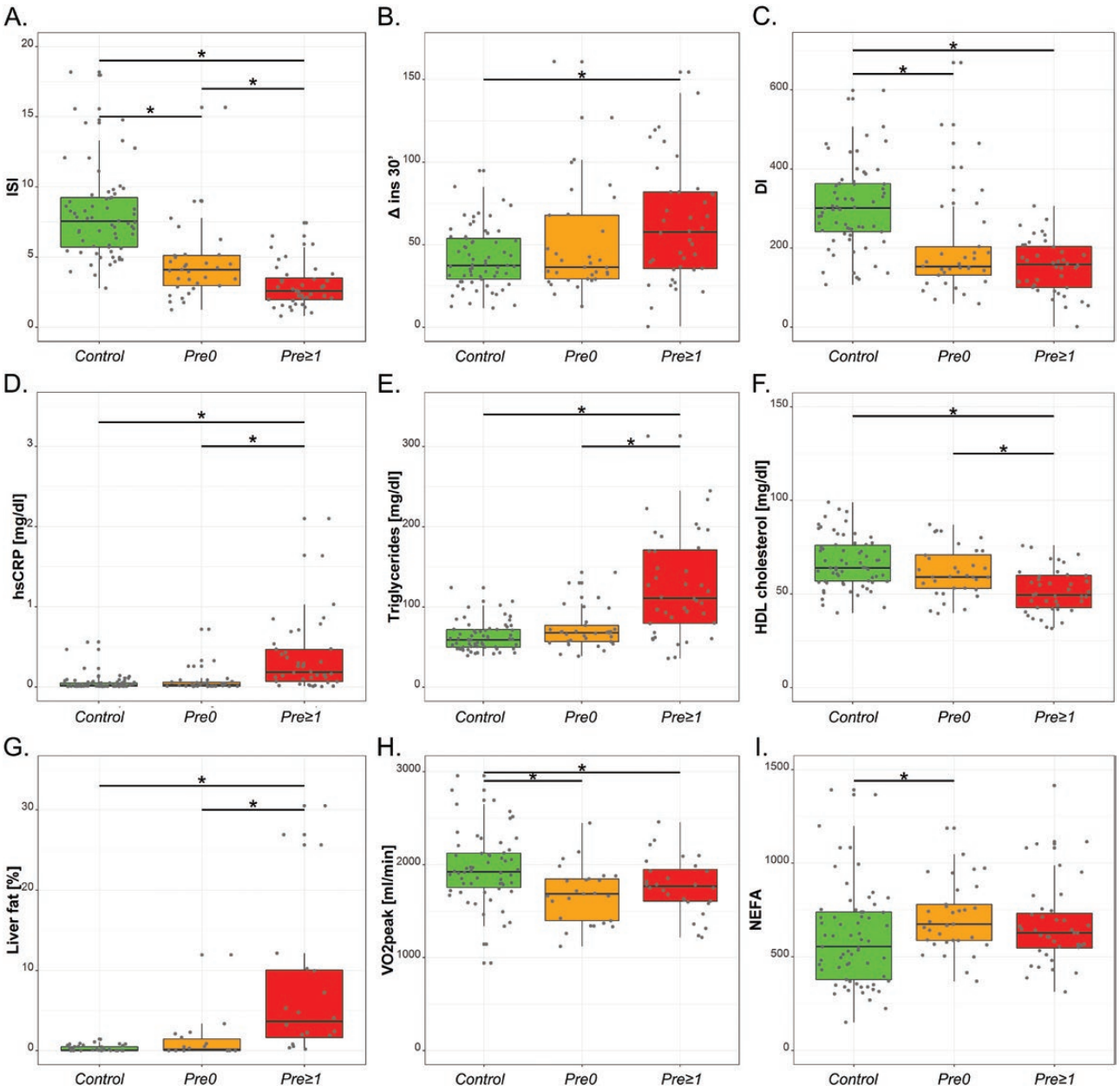

Figure 1. Main differences between Control, Pre0, and Pre≥1 in the PPSDiab study. *Indicates pairwise significance in Dunn's post-hoc procedure (after Kruskal-Wallis test).

Insulin sensitivity and secretion also differed between the groups. Pre0 demonstrated a lower insulin sensitivity index (ISI) than Control, equivalent early insulin secretion (Δins 30'), and a reduced disposition index (DI) in the oGTT. Similarly, the beta cell adaptation index (8) was reduced. First-phase insulin release (FPIR) in the intravenous glucose tolerance test was comparable between Pre0 and Control, but the disposition index calculated from FPIR and ISI (ivDI) was again reduced in Pre0. Pre≥1 depicted a lower ISI and a higher early insulin secretion (Δins 30' and FPIR) than both other groups. Both DI and ivDI were reduced in Pre≥1 compared to Control but were equivalent to Pre0. The muscle insulin sensitivity index (9) was lower and the hepatic insulin resistance index (3) was higher in both Pre0 and Pre≥1 compared to Control. Among the different metabolic indices, ISI had the highest discriminatory power between Control and Pre0, albeit with overlapping 95% confidence intervals of the respective area under the receiver operating characteristic curve of a binary logistic regression model values (Table 4). The only differences between Control and Pre0, beyond glucose metabolism, were elevated NEFA and a reduced VO_2peak in Pre0. VO_2peak was also lower in Pre≥1 compared to Control (Table 3; Fig. 1).

Table 4. Discriminatory power of different metabolic indices between Control and Pre0, as well as Control/78 and Pre0/78 in the PPSDiab study

Index	AUC-ROC Control *vs* Pre0 (95% CI)	AUC-ROC Control/78 *vs* Pre0/78 (95% CI)
ISI	0.85 (0.76-0.94)	0.81 (0.68-0.94)
DI	0.77 (0.65-0.88)	0.71 (0.53-0.88)
Muscle Insulin Sensitivity Index	0.64 (0.52-0.76)	0.65 (0.50-0.81)
Hepatic Insulin Resistance Index	0.73 (0.63-0.84)	0.73 (0.60-0.87)
Beta Cell Adaptation Index	0.77 (0.66-0.87)	0.73 (0.57-0.88)

Abbreviations: AUC-ROC, area under the receiver operating characteristic curve of a binary logistic regression model; CI, confidence interval.

To exclude type 1 diabetes in Pre0, we measured glutamic acid decarboxylase 65-kilodalton isoform, islet antigen 2, and ZnTF8 antibodies. Two women (6%) were marginally positive for 1 of the 3 antibodies, but these women were metabolically comparable to the rest of the group, showed no other signs of autoimmune diabetes, and were antibody-negative at follow-up study visits (data not shown). To also exclude GCK maturity-onset diabetes of the young (15) in Pre0, we sequenced the GCK gene. In this analysis, we found no known maturity-onset diabetes of the young (MODY) mutations.

To foster a sensitivity analysis, we further limited the Pre0 group to a waist circumference of less than 78 cm (instead of 88 cm in the NCEP ATP3 score) and compared these women to the corresponding Control group (waist < 78 cm). The results were the same as in our previous analysis with a waist cutoff of 88 cm, except that VO_2peak no longer differed between Pre0/78 and Control/78 (Table 5; Fig. 2).

Whitehall II study

For validation of the findings from the PPSDiab study in a cohort more representative of the general population and with more incident cases of T2DM, we analyzed data from the Whitehall II study. Here, we divided the incident cases of T2DM into 2 groups. The first group, Diab0, included all cases with 0 non-hyperglycemia points for metabolic syndrome. The second group, Diab≥1, consisted of all cases with at least 1 non-hyperglycemia point for metabolic syndrome. In study phases 3, 5, 7, and 9 combined, 412 cases of incident T2DM occurred, of which 62 (15%) belonged to Diab0 and, correspondingly, 350 (85%) belonged to Diab≥1. The proportion of Diab0 was highest in phase 3 (28 out of 146 cases; 19%), in which the participant age was 50.0 ± 6.0 years and lowest in phase 9 (6 out of 75 cases; 8%), in which the participant age was 65.5 ± 5.9 years (Table 6; Fig. 3).

We further characterized the metabolic phenotype of the 62 cases in Diab0 by matching each case by age (±2 years), sex, and study phase to 4 normoglycemic control subjects with 0 points for metabolic syndrome. A comparison with these control subjects then revealed equal values in the metabolic syndrome components waist circumference, systolic and diastolic blood pressure, triglycerides, HDL cholesterol, and interleukin 6. The mean BMI was even lower in Diab0 than in Control. In contrast, ISI and homeostatic model assessment of beta cell function (HOMA1-B) were both lower in Diab0 (Table 7).

Discussion

We demonstrate in 2 complementary human studies that T2DM and its prediabetic precursor states occur unrelated to metabolic syndrome in a relevant proportion of affected individuals. In a cohort of primarily prediabetic young women after GDM, 45% fell into this category while in the more diverse and older Whitehall II cohort, in which we examined incident T2DM, the proportion was 15%. These individuals reached 0 non-hyperglycemia points in the NCEP ATP3 score and, moreover, were comparable to healthy control subjects in a broad panel of metabolic syndrome components.

Metabolic syndrome, with its components dyslipidemia, hypertension, insulin resistance, low-grade inflammation, and ectopic lipid deposition, undoubtedly plays a role in the development of T2DM and other cardiometabolic diseases in many cases (18,19). This is reflected, for example, in the Pre≥1 group of the PPSDiab cohort. However, impaired glucose metabolism and T2DM also seem to occur in a different pathophysiologic context unrelated to metabolic syndrome, as we saw in Pre0, Pre0/78, and Diab0.

For these groups, autoimmune diabetes would be a plausible alternative explanation, but we would exclude that as the predominant pathophysiology. In the PPSDiab study, we base this conclusion on antibody testing, which was not performed in the Whitehall II study. Yet, the proportion of cases in the Diab0 group of Whitehall II was much larger than what we would have expected for autoimmune diabetes. This expectation derives from a recent publication by Thomas et al (20) that estimated the frequency of type 1 diabetes in different age groups of UK Biobank participants based on genetic evidence. In the age range of the Whitehall II study, the estimate was 1% to 2% of type 1 among incident cases of diabetes, not 15%, as we determined it for the Diab0 group. In the PPSDiab

Table 5. Characteristics of Control/78 and Pre0/78 in the PPSDiab study

	Control/78	Pre0/78	*P*-value[a]
N	48	20	
Glucose tolerance status			
NGT	48 (100%)	—	<0.0001
IFG		10 (50.0%)	
IGT		6 (30.0%)	
IFG and IGT		3 (15.0%)	
Diabetes		1 (5.0%)	
Age, years	35.5 ± 4.5	35.5 ± 3.5	0.83
Time since delivery, months	9.5 ± 2.7	9.4 ± 3.0	0.66
Hormonal contraception			
No	39 (81.3%)	15 (75.0%)	0.87
Gestagene	7 (14.6%)	4 (20.0%)	
Combination	2 (4.2%)	1 (5.0%)	
Active smoker	1 (2.1%)	0 (0%)	0.71
Systolic BP, mmHg	110.1 ± 7.7	110.6 ± 8.3	0.64
Diastolic BP, mmHg	68.1 ± 6.3	69.1 ± 7.1	0.67
BMI, kg/m^2	21.2 ± 1.5	21.2 ± 2.3	0.44
Waist circumference, cm	71.9 ± 3.6	71.8 ± 2.8	0.65
Body fat, %	26.1 ± 5.3	25.2 ± 5.2	0.49
hsCRP, mg/dL	0.02 (0.01-0.06)	0.02 (0.01-0.08)	0.99
Liver fat, % (n = 40)	0.07 (0-0.53)	0 (0-0.51)	0.55
Intra-abdominal fat	1.0 (0.8-1.4)	1.0 (0.9-1.3)	0.74
V0$_2$peak, mL/min (n = 58)	1856 (1701-2236)	1825 (1663-1877)	0.18
Triglycerides, mg/dL	60.5 (50.5-72.0)	65.0 (55.5-69.0)	0.69
HDL cholesterol, mg/dL	66.0 (56.0-74.5)	63.5 (57.5-78.5)	0.99
NEFA, µmol/L	604 (437-765)	756 (588-912)	0.03
Fasting PG, mmol/L	4.9 (4.6-5.1)	5.7 (5.3-5.8)	<0.0001
2-h PG, mmol/L	4.8 (4.4-5,9)	7.3 (6.4-88)	<0.0001
Fasting insulin, µU/ml	4.7 (3.8-6.0)	7.3 (5.1-11.9)	0.0039
ISI	7.8 (6.0-9.6)	4.4 (3.2-6.7)	0.0002
$^{\Delta}$ins 30’	36.6 (28.5-50.1)	37.6 (28.4-64.0)	0.57
DI ($^{\Delta}$ins 30’ × ISI)	302 (243-362)	153 (127-326)	0.01
$^{\Delta}$C-peptid 30’, ng/mL	3.8 (3.1-4.8)	4.0 (3.4-5.9)	0.1894
Beta Cell Adaptation Index	31.0 (23.2-36.4)	17.3 (12.1-31.9)	0.0033
Hepatic Insulin Resistance Index	228.4 (181.2-330.2)	341.0 (267.0-511.7)	0.0026
Muscle Insulin Sensitivity Index (n = 64)	16.8 (11.9-29.2)	12.1 (8.2-18.4)	0.0605
FPIR (n = 39)	1.8 (1.2-2.3)	1.5 (1.1-2.3)	0.74
ivDI (FPIR × ISI; n = 39)	14.9 (9.5-18.3)	5.9 (3.8-8.6)	0.001

[a]*P*-value of Mann-Whitney-U test for metric and of chi-square or Fisher’s exact test for categorical variables. For details on metabolic indices, see Table 1.
Abbreviations: BMI, body mass index; BP, blood pressure; $^{\Delta}$ins 30’, rise of serum insulin during the first 30 minutes of the oral glucose tolerance test; DI, disposition index calculated from oral glucose tolerance test; FPIR, first-phase insulin release in the intravenous glucose tolerance test; hsCRP, high-sensitivity c-reactive protein; ISI, insulin sensitivity index; ivDI, disposition index calculated from FPIR and ISI; NEFA, nonesterified fatty acids; PG, plasma glucose; VO$_2$peak, peak oxygen uptake in cardiopulmonary exercise testing.

study, we also excluded GCK-MODY in Pre0, because of the recruitment of the participating women through their previous GDM, which occasionally is confused with GCK-MODY (21).

Consistently, both studies showed that T2DM unrelated to metabolic syndrome includes a component of insulin resistance and of impaired insulin secretion. In the PPSDiab study, in which we tested this in more detail, the insulin sensitivity of the Pre0 group fell in the middle between Control and Pre≥1, whereas early/first-phase insulin secretion was comparable to Control. This combination resulted in a DI and a Beta Cell Adaptation Index in Pre0 that was as low as in Pre≥1, which suggests inadequate adaptation of insulin secretion to the prevailing insulin resistance in this group. In the Whitehall II study, insulin resistance co-occurred with a reduced HOMA1-B in Diab0. HOMA1-B was the only available measure of insulin secretion in this study.

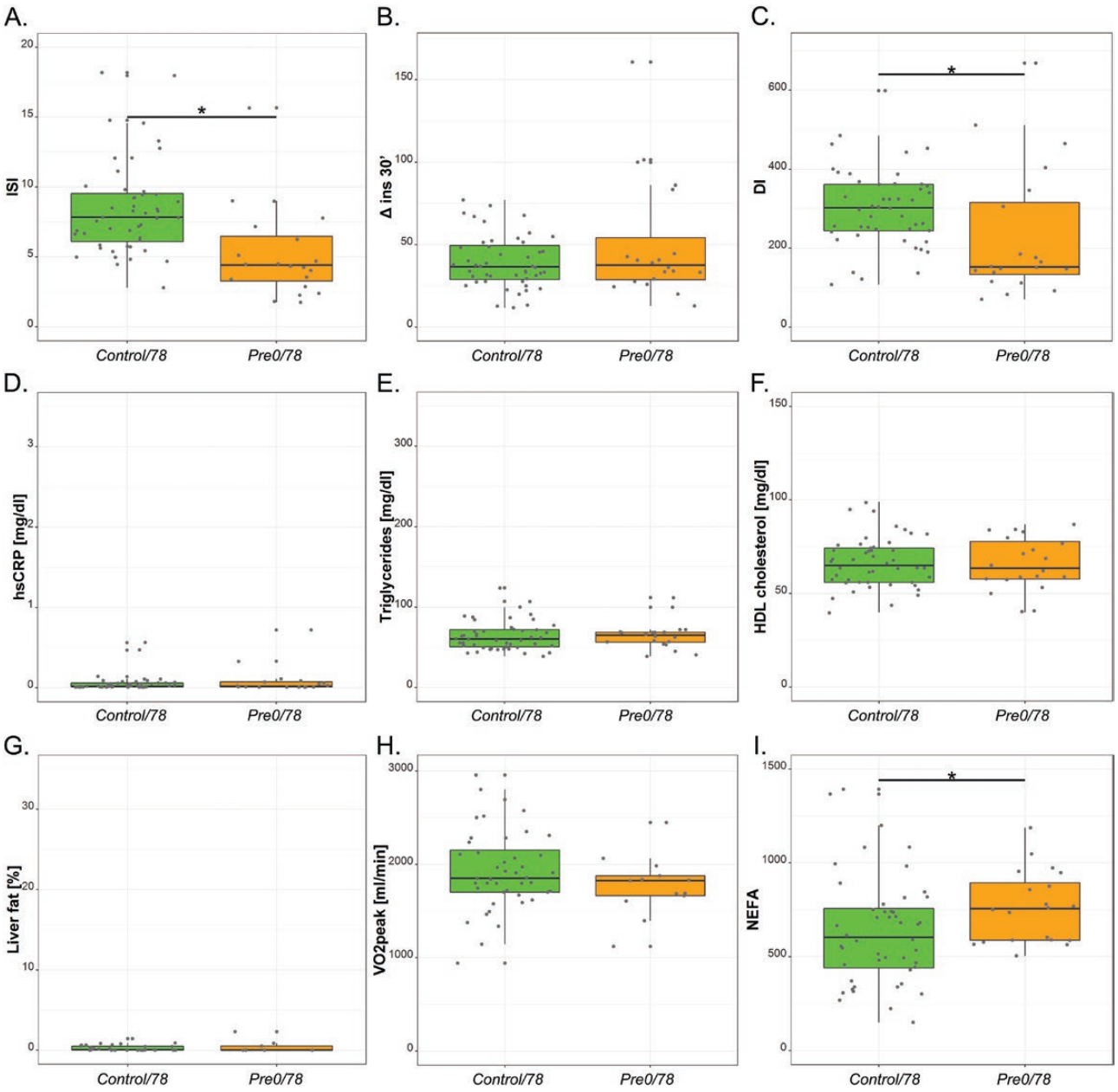

Figure 2. Main differences between Control/78 and Pre0/78 in the PPSDiab study. *Indicates significance in the Mann-Whitney U test.

Table 6. Incident cases of T2DM in the Whitehall II study

Study phase	3	5	7	9
Participants included	7655	3970	3344	2836
Age, years	50.0 ± 6.0	55.5 ± 6.0	60.8 ± 5.9	65.5 ± 5.9
Female	2358 (30.8%)	1209 (30.5%)	964 (28.8%)	774 (27.3%)
Incident diabetes	146	87	104	75
Diab0	28 (19%)	14 (16%)	14 (13%)	6 (8%)
Age	52.2 ± 6.9	59.0 ± 5.0	64.4 ± 6.4	67.6 ± 5.4
Female	12 (42.9%)	5 (57.1%)	5 (35.7%)	0
Diab≥1	118 (81%)	73 (84%)	90 (87%)	69 (92%)
Age	53.1 ± 5.6	57.8 ± 6.1	62.3 ± 6.1	66.5 ± 6.3
Female	31 (26.3%)	24 (32.9%)	24 (26.7%)	10 (14.5%)

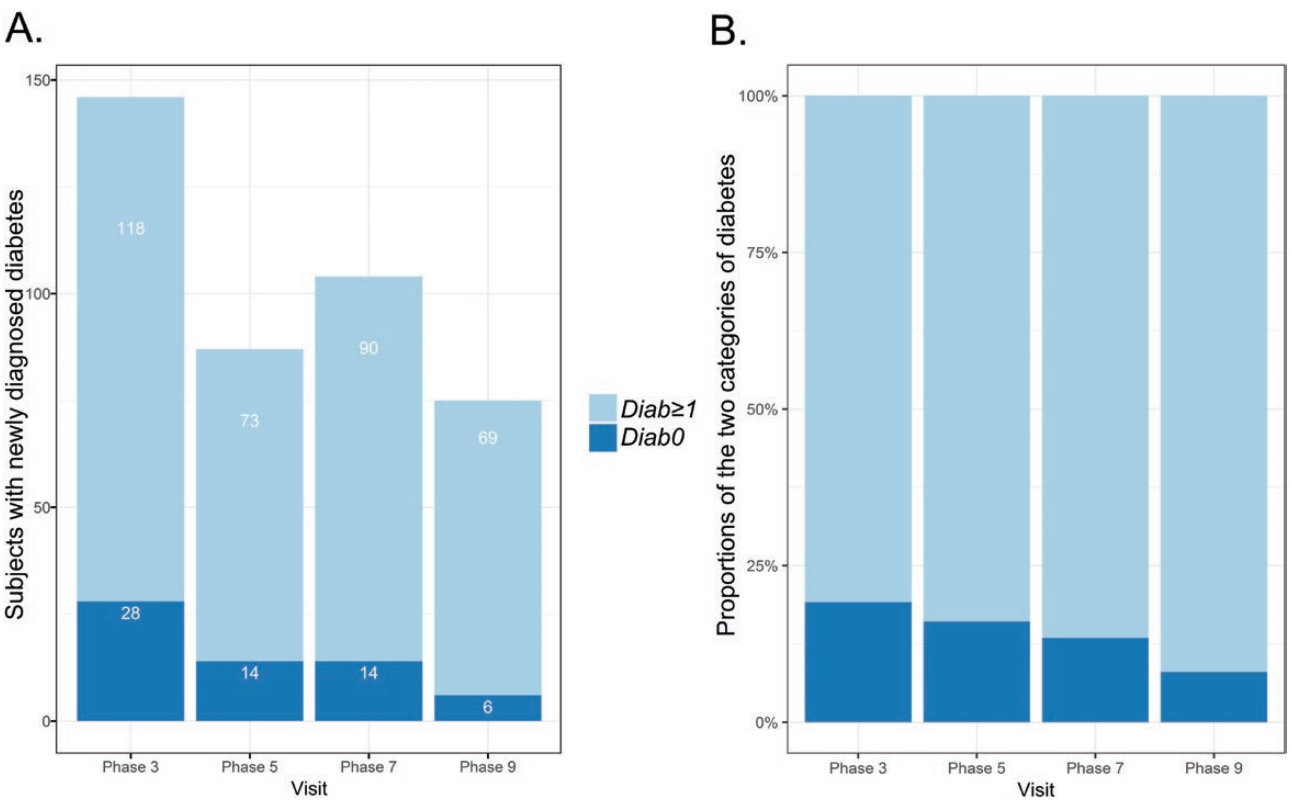

Figure 3. Incident cases of T2DM in the Whitehall II Study by study phase. (A) Absolute number of subjects in the Diab0 and the Diab≥1 group. B. Proportions of subjects in the Diab0 and the Diab≥1 group. Participant age at phase 3 was 50.0 ± 6.0, at phase 5 55.5 ± 6.0, at phase 7 60.8 ± 5.9, and at phase 9 65.5 ± 5.9 years.

Table 7. Case control comparison within the Whitehall II study

	Diab0	*Control*	*P*-value[a]
n	62	248	
Age, years	58.0 ± 8.5	58.1 ± 8.2	0.98
Female	25 (40.3%)	100 (40.3%)	1.00
Smoker	5 (8.1%)	15 (5.7%)	0.50
BMI, kg/m^2	23.2 ± 2.7	24.1 ± 2.8	0.03
Waist circumference, cm	79.6 ± 10.0	81.2 ± 10.2	0.24
Systolic BP, mmHg	112.6 ± 9.1	114.2 ± 8.8	0.22
Diastolic BP, mmHg	69.5 ± 8.3	70.4 ± 7.6	0.48
Triglycerides, mg/dL	0.9 (0.7-1.2)	0.9 (0.7-1.2)	0.45
HDL cholesterol, mg/dL	1.7 ± 0.4	1.7 ± 0.4	0.87
IL6, pg/mL (missing = 22)	1.6 (1.0-2.0)	1.4 (0.9-1.8)	0.09
Fasting PG, mmol/L	5.3 (5.0-7.0)	4.9 (4.7-5.2)	<0.0001
	95 (90-126)	*88 (85-94)*	
2-h PG, mmol/L (missing = 17)	11.7 (11.1-12.9)	5.4 (4.6-6.1)	<0.0001
	211 (200-232)	*97 (83-110)*	
ISI (missing = 33)	4.6 (2.9-6.4)	9.1 (6.0-14,3)	<0.0001
HOMA1-B (missing = 20)	48.6 (26.6-72.3)	65.3 (44.3-96.7)	0.0004

Abbreviations: BP, blood pressure; BMI, body mass index; IL6, interleukin 6; PG, plasma glucose; ISI, insulin sensitivity index, calculated from timepoints fasting and 2 h (16); HOMA1-B, homeostatic model assessment of beta cell function (17).
[a]*P*-value of Wilcoxon signed rank test.

What causes insulin resistance unrelated to metabolic syndrome remains incompletely understood. Separate indices of muscle and hepatic insulin resistance suggested in the PPSDiab study that both of these major determinants of whole-body insulin sensitivity were affected. We observed a lower physical fitness in Pre0 that may contribute to this situation (5). However, in the Pre0/78 group of our sensitivity analysis, this was no longer the case, and in the Whitehall II study, data on physical fitness was not available. In contrast, plasma NEFA were consistently higher in Pre0 and Pre0/78 than in Control in the PPSDiab study. This finding could suggest insulin-resistant adipose tissue as one component of the pathophysiology unrelated to metabolic syndrome, as already seen by others (22).

The factors limiting insulin secretion in the case groups without metabolic syndrome have also not yet been determined. In the PPSDiab study, the Beta Cell Adaptation Index, which relies on C-peptide measurements, suggested that secretion is truly inadequate and argued against dissimilarities in hepatic insulin extraction explaining the observed findings. However, the absence of very early time points of C-peptide measurements during the oGTT limited our ability to fully describe secretion kinetics. The secretion-limiting factors may include the genetic background and the lipotoxicity caused by the elevated NEFA levels. Additionally, these case groups may overlap with some of the insulinopenic subtypes of T2DM, described recently in population-based studies (23,24). Furthermore, an overlap of the different pathophysiologies (ie, first without and later with metabolic syndrome) may occur during the long-lasting process of T2DM development in an individual. This occurrence may also explain the decline in the proportion of cases without components of metabolic syndrome with advancing age, as we observed it between the 2 studies and also within the Whitehall II study.

The strengths of this work result from the 2 complementary cohorts examined and the deep phenotyping available in one of the studies. Its main limitation is the cross-sectional, descriptive design of the analyses. With this approach, we could not clarify cause-effect relationships as well as the dynamic processes occurring during T2DM development over time. Additionally, we did not measure insulin sensitivity with the gold-standard technique of a hyperinsulinemic clamp but estimated it from the oGTT, which could have led to deviances. However, in a previous study, we confirmed a high correlation of both approaches for the PPSDiab study (4). Finally, the cohorts examined included primarily individuals of European descent, thus limiting the validity of our findings to this population.

Conclusions

Our results suggest a second, common pathophysiology of T2DM that is unrelated to metabolic syndrome. If confirmed by others, strategies for the prevention and treatment of T2DM should deviate from the current, almost exclusive focus on metabolic syndrome. Instead, subgroup-specific, targeted interventions and medications should be applied.

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Data Availability: Data from the PPSDiab study are available from the corresponding author upon reasonable request. Whitehall II data are available to bona fide researchers for research purposes. Please refer to the Whitehall II data sharing policy at http://www.ucl.ac.uk/whitehallII/data- sharing.

References

1. Taylor R, Holman RR. Normal weight individuals who develop type 2 diabetes: the personal fat threshold. *Clin Sci (Lond)*. 2015;**128**(7):405-410.
2. Preliminary estimates of the prevalence of selected underlying health conditions among patients with coronavirus disease 2019—United States, February 12-March 28, 2020. *MMWR Morb Mortal Wkly Rep*. 2020;**69**(13):382-386.
3. Bellamy L, Casas JP, Hingorani AD, Williams D. Type 2 diabetes mellitus after gestational diabetes: a systematic review and meta-analysis. *Lancet*. 2009;**373**(9677):1773-1779.

4. Rottenkolber M, Ferrari U, Holland L, et al. The diabetes risk phenotype of young women with recent gestational diabetes. *J Clin Endocrinol Metab*. 2015;**100**(6):E910-E918.
5. Gar C, Rottenkolber M, Grallert H, et al. Physical fitness and plasma leptin in women with recent gestational diabetes. *PLoS One*. 2017;**12**(6):e0179128.
6. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care*. 1999;**22**(9):1462-1470.
7. Kahn SE, Prigeon RL, McCulloch DK, et al. Quantification of the relationship between insulin sensitivity and beta-cell function in human subjects: evidence for a hyperbolic function. *Diabetes*. 1993;**42**(11):1663-1672.
8. Ahrén B, Pacini G. Impaired adaptation of first-phase insulin secretion in postmenopausal women with glucose intolerance. *Am J Physiol*. 1997;**273**(4):E701-E707.
9. Abdul-Ghani MA, Matsuda M, Balas B, DeFronzo RA. Muscle and liver insulin resistance indexes derived from the oral glucose tolerance test. *Diabetes Care*. 2007;**30**(1):89-94.
10. Borg E, Kaijser L. A comparison between three rating scales for perceived exertion and two different work tests. *Scand J Med Sci Sports*. 2006;**16**(1):57-69.
11. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res*. 1988;**16**(3):1215.
12. Tabák AG, Jokela M, Akbaraly TN, Brunner EJ, Kivimäki M, Witte DR. Trajectories of glycaemia, insulin sensitivity, and insulin secretion before diagnosis of type 2 diabetes: an analysis from the Whitehall II study. *Lancet*. 2009;**373**(9682):2215-2221.
13. Classification and diagnosis of diabetes: *Standards of Medical Care in Diabetes—2020*. *Diabetes Care*. 2020;**43**(Suppl 1):S14-S31.
14. Alberti KG, Eckel RH, Grundy SM, et al.; International Diabetes Federation Task Force on Epidemiology and Prevention; Hational Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; International Association for the Study of Obesity. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation*. 2009;**120**(16):1640-1645.
15. Rudland VL, Hinchcliffe M, Pinner J, et al. Identifying glucokinase monogenic diabetes in a multiethnic gestational diabetes mellitus cohort: new pregnancy screening criteria and utility of HbA1c. *Diabetes Care*. 2016;**39**(1):50-52.
16. DeFronzo RA, Matsuda M. Reduced time points to calculate the composite index. *Diabetes Care*. 2010;**33**(7):e93.
17. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. *Diabetes Care*. 2004;**27**(6):1487-1495.
18. Caputo T, Gilardi F, Desvergne B. From chronic overnutrition to metaflammation and insulin resistance: adipose tissue and liver contributions. *FEBS Lett*. 2017;**591**(19):3061-3088.
19. Shulman GI. Ectopic fat in insulin resistance, dyslipidemia, and cardiometabolic disease. *N Engl J Med*. 2014;**371**(12): 1131-1141.
20. Thomas NJ, Jones SE, Weedon MN, Shields BM, Oram RA, Hattersley AT. Frequency and phenotype of type 1 diabetes in the first six decades of life: a cross-sectional, genetically stratified survival analysis from UK Biobank. *Lancet Diabetes Endocrinol*. 2018;**6**(2):122-129.
21. Weng J, Ekelund M, Lehto M, et al. Screening for MODY mutations, GAD antibodies, and type 1 diabetes-associated HLA genotypes in women with gestational diabetes mellitus. *Diabetes Care*. 2002;**25**(1):68-71.
22. Acosta JR, Douagi I, Andersson DP, et al. Increased fat cell size: a major phenotype of subcutaneous white adipose tissue in non-obese individuals with type 2 diabetes. *Diabetologia*. 2016;**59**(3):560-570.
23. Ahlqvist E, Storm P, Käräjämäki A, et al. Novel subgroups of adult-onset diabetes and their association with outcomes: a data-driven cluster analysis of six variables. *Lancet Diabetes Endocrinol*. 2018;**6**(5):361-369.
24. Stidsen JV, Henriksen JE, Olsen MH, et al. Pathophysiology-based phenotyping in type 2 diabetes: A clinical classification tool. *Diabetes Metab Res Rev*. 2018;**34**(5):e3005.