Subphenotype-dependent benefits of bariatric surgery for individuals at risk for type 2 diabetes

Running title: Bariatric surgery in prediabetes subphenotypes

Leontine Sandforth[1,2,3*], Violeta Raverdy[4,5*], Arvid Sandforth[1,2,3], Pierre Bauvin[6], Estelle Chatelain[7], Helene Verkindt[4,5], Geltrude Mingrone[8,9,10], Caterina Guidone[9], Ornella Verrastro[8], Karin Zhou[1,2,3], Rami Archid[11], André Mihaljevic[11], Robert Caiazzo[4,5], Gregory Baud[4,5], Camille Marciniak[4,5], Mikael Chetboun[4,5], Marlene Ganslmeier[1,2,3], Vitória Minelli Faiao[1,2,3], Martin Heni[12,13], Louise Fritsche[2,3], Anja Moller[1,2,3], Konstantinos Kantartzis[1,2,3], Andreas Peter[2,3,12], Rainer Lehmann[2,3,12], Robert Wagner[3,14,15], Katsiaryna Prystupa[3,14,15], Andreas Fritsche[1,2,3], Norbert Stefan[1,2,3], Hubert Preissl[1,2,3,16], Andreas L Birkenfeld[1,2,3,17], Reiner Jumpertz von Schwartzenberg[1,2,3,18,19*+], François Pattou[4,5*+]

Affiliations:

[1]Internal Medicine IV, Endocrinology, Diabetology and Nephrology, University Hospital of Tübingen, Tübingen, Germany

[2]Institute of Diabetes Research and Metabolic Diseases (IDM) of the Helmholtz Center Munich, Tübingen, Germany

[3]German Center for Diabetes Research (DZD), Neuherberg, Germany

[4]University Lille, INSERM, CHU Lille, Institut Pasteur de Lille, UMR 1190 Translational Research for Diabetes, European Genomic Institute for Diabetes, Lille, France

[5]CHU Lille, Integrated Center for Obesity, General and Endocrine Surgery, Lille, France

[6]University Lille, Inserm, CHU Lille, Institut Pasteur de Lille, U1190-EGID, Lille, France

[7]University Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, US 41-UAR 2014-PLBS, F-59000 Lille, France

[8]Catholic University of the Sacred Heart, Department of Internal Medicine, Rome, Italy

[9]Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy

[10]Division of Diabetes & Nutritional Sciences, School of Cardiovascular and Metabolic Medicine & Sciences, King's College London, London, United Kingdom

[11]Department of General, Visceral, and Transplant Surgery, University Hospital of Tübingen, Tübingen, Germany

[12]Institute for Clinical Chemistry and Pathobiochemistry, Department for Diagnostic Laboratory Medicine, University Hospital of Tübingen, Hoppe-Seyler-Str. 3, 72076 Tübingen, Germany

[13]Division of Endocrinology and Diabetology, Department of Internal Medicine I, University Hospital Ulm, Ulm, Germany.

[14]Institute for Clinical Diabetology, German Diabetes Center, Leibniz Institute for Diabetes Research at Heinrich Heine University Düsseldorf (DDZ), Düsseldorf, Germany

[15]Department of Endocrinology and Diabetology, Medical Faculty, Heinrich Heine University, University Hospital, Düsseldorf, Germany

[16]Institute of Pharmaceutical Sciences, Department of Pharmacy and Biochemistry; Interfaculty Centre for Pharmacogenomics and Pharma Research at the Eberhard Karls University Tübingen, Tübingen, Germany

[17]School of Cardiovascular and Metabolic Medicine & Sciences, King's College London, London, UK

[18]Cluster of Excellence EXC 2124 "Controlling Microbes to Fight Infections" (CMFI), University of Tübingen, Tübingen, Germany

[19]M3 Research Center, Malignom, Metabolome, Microbiome, Otfried-Müller-Str. 37, 72076 Tübingen, Germany

[*]These authors contributed equally

[+]Corresponding authors

Authors' email addresses:

Leontine Sandforth: leontine.sandforth@med.uni-tuebingen.de
Violeta Raverdy: violeta.raverdy@univ-lille.fr
Arvid Sandforth: arvid.sandforth@med.uni-tuebingen.de
Pierre Bauvin: pierre.bauvin.imag@gmail.com
Estelle Chatelain: estelle.chatelain@univ-lille.fr
Helene Verkindt: helene.verkindt@chu-lille.fr
Geltrude Mingrone: geltrude.mingrone@unicatt.it

Caterina Guidone: caterina.guidone@policlinicogemelli.it
Ornella Verrastro: ornella.verrastro@gmail.com
Karin Zhou: karinzhou97@gmail.com
Rami Archid: rami.archid@diak-stuttgart.de
André Mihaljevic: andre.mihaljevic@med.uni-tuebingen.de
Robert Caiazzo: robert.caiazzo@chu-lille.fr
Gregory Baud: gregory.baud@chu-lille.fr
Camille Marciniak: camille.marciniak@chu-lille.fr
Mikael Chetboun: mikael.chetboun@chu-lille.fr
Marlene Ganslmeier: marlene.ganslmeier@med.uni-tuebingen.de
Vitória Minelli Faiao: vitoria.minelli-faiao@student.uni-tuebingen.de
Martin Heni: martin.heni@uniklinik-ulm.de
Louise Fritsche: louise.fritsche@googlemail.com
Anja Moller: anja.moller@med.uni-tuebingen.de
Konstantinos Kantartzis: konstantinos.kantartzis@med.uni-tuebingen.de
Andreas Peter: andreas.peter@med.uni-tuebingen.de
Rainer Lehmann: rainer.lehmann@med.uni-tuebingen.de
Robert Wagner: robert.wagner@ddz.de
Katsiaryna Prystupa: katsiaryna.prystupa@ddz.de
Andreas Fritsche: andreas.fritsche@med.uni-tuebingen.de
Norbert Stefan: norbert.stefan@med.uni-tuebingen.de
Hubert Preissl: hubert.preissl@uni-tuebingen.de
Andreas Birkenfeld: andreas.birkenfeld@med.uni-tuebingen.de
Francois Pattou: francois.pattou@univ-lille.fr
Reiner Jumpertz-von Schwartzenberg: reiner.jumpertz-vs@med.uni-tuebingen.de

Corresponding authors:
Reiner Jumpertz-von Schwartzenberg: reiner.jumpertz-vs@med.uni-tuebingen.de, phone: +49 (0) 7071 29 80.
Francois Pattou: francois.pattou@univ-lille.fr, phone: +33 (0)3 20 62 69 63

Twitter summary
Individuals at risk for type 2 diabetes (T2D) respond differently to bariatric surgery (BS) according to novel clusters (Tübingen Clusters), of which high-risk clusters benefitted most from BS.

Key words: Obesity, Prediabetes, Bariatric Surgery, Remission, Type 2 Diabetes
Word count: 3993 words, 3 Figures, 1 Table

Abstract

Objective

Bariatric surgery (BS) is an effective treatment option for individuals with obesity and type 2 diabetes (T2D). However, whether outcomes in subtypes of individuals at risk for T2D and/or comorbidities (Tübingen Clusters) differ, is unknown. Of these, clusters 5 and 6 (C5, C6) are high-risk clusters for developing T2D and/or comorbidities, while cluster 4 (C4) is a low-risk cluster. We investigated BS outcomes, hypothesizing high-risk clusters benefit most due to great potential for metabolic improvement.

Research Design and Methods

We allocated participants without T2D but at risk for T2D, defined by elevated BMI, to the Tübingen Clusters. Participants had normal glucose regulation or prediabetes according to American Diabetes Association criteria. Two cohorts underwent BS: A discovery (Lille, France) and a replication cohort (Rome, Italy). A control cohort (Tübingen, Germany) received behavioral modification counseling. Main outcomes included glucose regulation and prediabetes remission.

Results

In the discovery cohort, 15.0% of participants (n=121) were allocated to C4, 22.3% (n=180) to C5, and 62.4% (n=503) to C6. Relative body weight loss was similar between all clusters, however C5 most strongly reduced insulin resistance and improved beta-cell function. Prediabetes remission rate was lowest in low-risk C4 and highest in high-risk C5. Individuals from high-risk clusters changed to low-risk clusters in all BS cohorts but not in the control cohort.

Conclusions

Participants in C5 had the highest benefit from BS in terms of improvement in insulin resistance, beta-cell function and prediabetes remission. This novel classification might help identify individuals who will benefit specifically from BS.

Highlights

- **Why did we undertake this study?** Today, prediction of the metabolic benefit of bariatric surgery (BS) for the individual without type 2 diabetes (T2D) is difficult. This study aimed at improving prediction of metabolic improvements in people from different risk strata without T2D.
- **What is the specific question(s) we wanted to answer?** We asked the question how individuals from different risk strata for T2D (Tübingen Clusters) respond to BS.
- **What did we find?** High-risk clusters had highest prediabetes remission rates and strongest reduction of liver fat. Furthermore, high-risk turned to low-risk clusters by majority after BS as opposed to individuals with behavioral modification only.
- **What are the implications of our findings?** These findings might help understand mechanisms of prediabetes remission after BS and help identify individuals specifically benefitting from BS.

Introduction

Worldwide, 2.5 billion adults are affected by overweight and obesity.(1) Many of these individuals develop type 2 diabetes (T2D) which ranges among the leading causes of death globally.(2) Most individuals have already established T2D-associated comorbidities such as nephropathy or macrovascular disease at the time of diagnosis. (3) Therefore, it is of utmost importance to improve T2D prevention and treat individuals earlier in the course of metabolic disease, specifically in prediabetes, since lifetime T2D risk in people aged >35 years with prediabetes is >70%.(4) Bariatric surgery (BS) is a well-established therapy to decelerate disease progression of obesity-associated sequelae such as cardiovascular events, renal disease or mortality via body weight reduction. It has been shown to improve glycemic control in people with T2D and even promote T2D remission. A recent data-driven classification of individuals with T2D has defined five clusters that show different disease progression patterns as well as diverging risks of diabetes complications.(5) Importantly, T2D clusters benefit from BS to different extents and in differential manners.(6) Subjects from the “severe insulin-resistant diabetes” (SIRD) cluster benefitted more from BS both in terms of T2D remission and renal function compared to the remaining clusters.

However, complications such as nephropathy can occur even before the onset of T2D.(3) Thus, it is important to further characterize and understand sub-phenotypes and treatment responses before T2D onset. In individuals at risk for T2D, defined by prediabetes and/or history of gestational diabetes and/or familial risk for T2D and/or elevated BMI, data-driven clusters differing in T2D risk and related complications have been identified. Clustering variables included anthropometrics, glucose and insulin measures from oral glucose tolerance tests (OGTTs), as well as fasting lipid levels.(7)

From these six clusters, cluster 4 (C4), 5 (C5) and 6 (C6) are linked with obesity, and

3 (C3), C5 and C6 showed a high risk to develop T2D and/or complications. C5 is characterized by high liver fat content and insulin resistance and high cardiovascular risk ("high liver fat content and insulin resistance-related cluster"). C6 shows a high nephropathy risk and high insulin secretion despite a relatively low risk to develop T2D ("nephropathy risk and high insulin secretion-related cluster"). C4 however belongs to the low-risk clusters associated with severe obesity, having a low risk to develop T2D or related complications ("low risk obesity cluster"). An overview of these "*Tübingen Clusters*" is provided in **Supplementary Figure 1**. Similar to individuals with T2D, individuals with prediabetes can reduce insulin resistance via weight loss after BS and may thereby significantly reduce their elevated T2D risk, as has been shown previously.(8) While current guidelines recognize T2D as a comorbidity guiding clinical decision making about BS, prediabetes representing an independent risk factor for cardiovascular events, kidney disease or even mortality, is currently not recognized as relevant obesity-associated comorbidity, partially due to its heterogeneity.(2,9) To account for this heterogeneity and to evaluate therapeutic responses to BS, participants with prediabetes were assigned to the *Tübingen Clusters*. In this study, we aim at examining the impact of cluster-specific impact from BS on glucose regulation parameters, prediabetes remission and cluster change in two cohorts of individuals with elevated risk for T2D undergoing BS and a control cohort.(10) We hypothesized that high-risk clusters benefit most from BS due to their potential to improve a previously deleterious metabolic state.

Research Design and Methods

Study design and participants

In this multi-cohort study, we investigate the post-operative outcomes of novel data-driven sub-phenotypes of individuals without T2D but at risk for T2D (defined by elevated BMI >27 kg/m^2) in two cohorts undergoing BS – the "A Biological Atlas of Severe Obesity" cohort (ABOS) in France (NCT01129297), the "Bariatric Surgery and Reactive Hypoglycemia" study cohort (Rome, Italy, NCT01581801) – and the "Clinical and Metabolic Characterization of Long-term Courses of Obesity Patients (AdipFollowup)" study cohort (Tübingen, Germany, NCT04375371) as control. ABOS participants were followed up after one and two years, Rome cohort participants for an average of 15.3 months [SD 4.5], and Control cohort participants for approximately ten years (128.9 months [SD 30.14]).

ABOS is an ongoing prospective study which aims at identifying determinants of outcomes of BS. ABOS participants without T2D (n=806) who underwent Roux-en-Y gastric bypass, sleeve gastrectomy or gastric banding between Jan 1, 2006 and Dec 12, 2017, were included in the present study (**Supplementary Figure 2**). Participant data were prospectively collected at the time of surgery, 1 and 2 years after surgery. A 75 g oral glucose tolerance test (OGTT) was performed at baseline and at follow up. A description of the laboratory assessments has been published previously.(11)

For the analysis of prediabetes remission, all individuals with prediabetes at baseline were included (n=423). Prediabetes status was defined at baseline based on fasting plasma glucose (PG) of 100 mg/dL to 125 mg/dL (5.6-6.9 mmol/L) or a 2-hour PG of 140-199 mg/dL (7.8-11.0 mmol/L) during OGTT or HbA1c of 5.7-6.4% (39-46 mmol/mol) according to American Diabetes Association (ADA) recommendations.(12) Glucose area under the curve during OGTT was determined using the trapezoidal rule ($AUC_{Glucose\ 0\text{-}120min}$).(13) Peripheral insulin sensitivity was estimated by the modified Matsuda index ($ISI_{Matsmod}$) according to the following formula:

$10000/\sqrt{([Glucose_{0min} \times Insulin_{0min}] \times [(Glucose_{0min}+Glucose_{30min}+Glucose_{120min})/3] \times [(Ins}$

$ulin_{0min}+Insulin_{30min}+Insulin_{120min})/3])$.(14,15) Insulin resistance was assessed by the Homeostastic Model Assessment for Insulin Resistance (HOMA-IR) which was calculated according to the following formula: $Insulin_{0min} \times Glucose_{0min}/22.5$,(16) the disposition index as measure of beta-cell function was calculated as the product of the C-peptidogenic index and Matsuda index (17) and the C-peptide/Glucose-Area under the curve using the trapezoidal rule as a proxy for insulin secretion ($AUC_{C\text{-}Peptide\ 0\text{-}30min}/AUC_{Glucose\ 0\text{-}30min}$).(18)

The independent replication cohort from Rome consisted of 60 individuals with obesity without T2D who were randomly assigned 1:1 to either Roux-en-Y gastric bypass or sleeve gastrectomy at the Catholic University School of Medicine in Rome (Italy) between December 2012 and December 2014.(19) A description of the analytic procedures of samples has been published previously.(19)

The independent control cohort from Tübingen, Germany, consisted of 46 individuals with obesity who received behavioral modification counseling for body weight reduction. This included 10 group sessions with nutrition, physical activity and lifestyle counseling over 6 months. A detailed description of the laboratory assessments has been published elsewhere.(20) Individuals of the Control cohort were retrospectively contacted for rephenotyping between January 27, 2020 and October 23, 2020.

The studies were reviewed and approved by the regional human ethics committees (Lille: Comité de Protection des Personnes Nord Ouest VI; Rome: Rome Catholic University Ethical Committee; Tübingen: Ethics committee at the Eberhard-Karls University of Tübingen) in accordance with national guidelines and the provisions of the Helsinki Declaration, as revised in 2000. All participants provided written informed consent to participate in the respective studies.

Clustering

Clinical clustering variables of the *Tübingen Clusters* were BMI, hip and waist circumference, fasting PG and insulin, 2-hour PG and insulin, fasting triglycerides and high-density lipoprotein cholesterol (HDL-C) levels.(7) The *Tübingen Clusters* were named after the first cohort in which they were described (Tübingen Family Study).(7,21) An online application for personal use or research purposes is accessible at https://prediabclusters.idm-tuebingen.org/.

Participants were assigned to C3, C4, C5 or C6 at baseline. Owing to the low number of participants assigned to C3 (ABOS: n=2, Control cohort: n=2), it was excluded from further analysis.

Surgery

All BS procedures were done laparoscopically, as described previously.(6)

Outcomes

Prediabetes remission was defined according to current ADA criteria for normal glucose regulation, as below the described cutoffs for prediabetes and without the use of glucose lowering drugs.(8,10,12)

Chronic kidney disease (CKD) was assessed based on estimated glomerular filtration rate (eGFR) calculated according to the Modification of Diet in Renal Disease formula.(22) General cardiovascular risk profile was estimated according to the Framingham sex-specific multivariable risk algorithm.(23) Liver biopsies were done as previously described.(24) The NAFLD Activity Score (NAS) was defined as the unweighted sum scores for steatosis (0-3), lobular inflammation (0-3) and ballooning.(25) Non-invasive tests (Fatty liver index (FLI), AST to platelet ratio index

(APRI), and NAFLD Fibrosis Score (NAFLD-FS)) were computed as described previously.(24)

Statistical analysis

Statistical analyses were performed using the R 2023.03.0+386 software. Data are presented as mean (CI), median (interquartile range), or n (%), unless otherwise specified. Between-group comparisons over time were analyzed using linear mixed-effects models, with participant as a random effect using the lme4 package, or cross-sectionally with two-way ANOVA with a post-hoc test for multiple comparisons (least significant difference), applying Bonferroni correction, or Wilcoxon signed-rank test or χ^2, as appropriate. The model included cluster, timepoint, and the interaction between the two as model terms and main outcomes were evaluated in models with BMI, age, sex and type of surgery as fixed effects, and in the case of insulin secretion additionally with insulin sensitivity.

Data and Resource Availability

The datasets generated or analyzed during the current study are not publicly available since they are subject to national data protection laws and restrictions imposed by the ethics committee to ensure data privacy of study participants. They can be applied for through an individual project agreement with the principal investigator of the respective university hospital.

Results

In all cohorts, the most abundant cluster was high-risk C6 (**Figure 1**). Participant anthropometric and metabolic characteristics of ABOS at baseline are summarized in

Table 1 and of remaining cohorts combined in **Supplementary Table 1**. Individuals

from low-risk C4 had lower BMI, liver fat content, triglycerides, insulin resistance and insulin secretion, glycemia and higher HDL-C compared to C5 and C6 at baseline.

Since *Tübingen Clusters* depend on modifiable metabolic measures, we hypothesized that BS leads to re-assignment of high-risk clusters to low-risk clusters. In ABOS and the Rome cohort, BS led to a switch from high-risk to low-risk clusters in most participants (**Figure 1A, B**). Equal fractions of C5 and C6 remained in high-risk clusters in ABOS, but nearly all subjects of C5 who did not convert to a low-risk cluster converted to C6 (**Figure 1A**). Finally, in the Control Cohort, most participants stayed in high-risk clusters and nearly half of the subjects of C4 turned to C6 after long-term follow up (**Figure 1C**).

Next, we investigated cluster-specific anthropometric outcomes of BS. One year after surgery, C5 and C6 had a higher BMI than C4 (1 year: C5 33.2 [SD 6.2]. *vs* C6 33.6 [SD 6.2]; p>0.99; C4 30.6 [SD 5.6]; each p *vs* C5 and C6 <0.001), while relative BMI change and body weight loss were similar between all clusters (body weight change: C4 27.9% [SD 11.0] *vs* C5 27.7 [SD 10.9] *vs* C6 27.9 [SD 11.1]; p=0.81; **Figure 2A**).

We further examined parameters of glucose regulation and insulin sensitivity. Initially, $AUC_{Glucose\ 0\text{-}120min}$ was highest in C5 and C6 (**Table 1**) and was reduced in both clusters after surgery with the most pronounced relative reduction in C5 *vs* the other clusters (**Figure 2B**). $AUC_{Glucose\ 0\text{-}120min}$ was lowest in C4 (**Table 1**), but $AUC_{Glucose\ 0\text{-}120min}$ did not change significantly one year after BS in this cluster (1 year: 761 min×mmol/L [SD 155]; p=0.9). PG levels after BS decreased to similar values between C6 and C4, but remained slightly higher in C5 *vs* C4 and C6 (**Figure 2G,I**). As expected, HOMA-IR was highest in C5 and lowest in C4 (p for each comparison <0.001, **Table 1**). After BS, C5 achieved the most pronounced reductions in HOMA-IR, with both C5 and C6 exhibiting stronger reductions in HOMA-IR than C4 (percentage ΔHOMA-IR C4: -

29.52%, C5: -53.82% *vs* C6: -53.00%; p C4 *vs* C5 or C6 <0.001, p C5 *vs* C6 >0.99;

Figure 2C). Similarly, $ISI_{Matsmod}$ increased strongest in C5 and C6 after BS (percentage $\Delta ISI_{Matsmod}$ C4: +66.44%, C5: +227.88%, C6: +150.80%; p for each comparison <0.001; **Figure 2D**). However, HOMA-IR and $ISI_{Matsmod}$ did not change significantly in C4 (**Figure 2C,D**). Disposition Index was highest in C4 and C6 at baseline (**Table 1**), while C4 was the only cluster not to increase beta-cell function significantly after BS (C4 +104.93% [SD 274.70], C5 +254.73% [SD 311.10], C6 +133.52% [SD 394.26], C4 *vs* C5 p=0.03, C4 *vs* C6 p=0.62, C5 *vs* C6 p=0.01; **Figure 2E**). $AUC_{C\text{-}Peptide\ 0\text{-}30min}/AUC_{Glucose\ 0\text{-}30min}$ was highest in C6 and lowest in C5 and C4 (**Table 1**). Only C5 increased insulin sensitivity-adjusted insulin secretion after BS, which was not observed in C4 and C6, while the latter is characterized by high insulin secretion (**Figure 2F, Supplementary Figure 5F**). Changes of the remaining glucose regulation parameters of the Rome cohort are shown in **Supplementary Figure 5**.

We next analyzed glucose regulation trajectories of C4, 5 and 6. Changes in glucose regulation status one year after BS are shown in **Figure 3A-C**. Despite exhibiting the lowest prediabetes prevalence, C4 had the lowest prediabetes remission rate of all participants who met prediabetes criteria at baseline, while C5 and C6 had the highest remission rate one year (C4 60% *vs* C5 77%; p=0.045; C4 *vs* C6 74%; p=0.09; **Figure 3D**) and two years after BS (C4 61% *vs* C5 82%; p=0.007; C4 *vs* C6 79%; p=0.047; **Figure 3E**).

To further understand underlying mechanisms contributing to prediabetes remission, we investigated anthropometric and metabolic parameters by prediabetes remission status (i.e. responder vs. non-responder). In all clusters, responders had more body weight loss than non-responders (C4 responders Δbody weight 35 kg [SD 5] *vs* non-responders 29 kg [SD 8], p group over time <0.05; C5 responders 38 kg [SD 3] *vs* non-responders 25 kg [SD 6], p group over time<0.001; C6 responders 42 kg [SD 2.5] *vs*

non-responders 26 kg [SD 5], p group over time <0.001; **Figure 3F**), indicating that

weight loss is important for prediabetes remission in all clusters. Insulin resistance/sensitivity (HOMA-IR and $ISI_{Matsmod}$) showed a stronger improvement in responders of C6 than in non-responders (**Figure 3G,H**), however improvement did not differ between non-responders and responders of C5 and C4. Furthermore, beta-cell function improved more strongly in responders from C5 than in non-responders but was not different between responders and non-responders in C4 (Disposition Index: C4 responders *vs* non-responders p group over time=0.07; C5 responders *vs* non-responders p group over time=0.02; C6 responders *vs* non-responders p group over time=0.33; **Figure 3J**). $AUC_{C\text{-}Peptide\ 0\text{-}30min}/AUC_{Glucose\ 0\text{-}30min}$ increased only in responders from C5.

Next, since glucose levels are critically regulated by the liver and partly depend on hepatic lipid content, local inflammation and fibrosis, we analyzed a subgroup of individuals who underwent initial and re-biopsy of the liver one year after BS (n=104). Here, C5 showed the highest liver steatosis severity and NAS but not Kleiner Liver Fibrosis Score at baseline (C4 *vs* C5, p<0.001; C4 *vs* C6, p<0.001; C5 *vs* C6, p=0.003, **Supplementary Figure 3A-C**). Most individuals from both C5 and C6 achieved a liver fat percentage reduction into the normal or near-normal range (**Supplementary Figure 3D**). C4 had a liver fat content corresponding to grade 1 macroscopic steatosis and reduced it by trend into the normal range. These findings were similar when assessed by non-invasive tests for steatosis and fibrosis (**Supplementary Figure 3E-G**). Scores for advanced metabolic dysfunction-associated steatotic liver disease (MASLD) like the APRI and FIB-4 were significantly reduced in non-responders of C6, while FIB-4 even increased in responders of C5 and C6 (**Supplementary Figure 4A-C**).

The highest prediabetes remission rate and the high conversion rate from high- to low-

risk cluster in C5 was also accompanied by the strongest reduction in relative

Framingham Risk Score (rFRS) although C5 had the highest age (**Supplementary Figure 6A**) and a slight increase of eGFR only in C5 and C6 (**Supplementary Figure 6B**). Glucose regulation trajectories of ABOS are shown in **Supplementary Figure 6C-D** and glucose regulation trajectories and prediabetes remission rate of the Rome and Control cohorts in **Supplementary Figure 7**.

Conclusions

In this study, we show that novel data-driven clusters of individuals at risk for T2D ("*Tübingen Clusters*") differ in their response to BS. As body weight loss was similar between all clusters, differences in the glucose regulation parameters were independent of differences in relative body weight loss. While high-risk clusters 5 and 6 ameliorated both insulin resistance and beta-cell function, specifically individuals with prediabetes from low-risk C4 did not benefit from BS to the same extent, which is demonstrated by its lower prediabetes remission rate. C4 achieved a moderate improvement in insulin resistance (despite basal HOMA-IR in the normal range), but neither responders nor non-responders from C4 improved insulin secretion or beta-cell function. Whether this was due to e.g. a lack of improving beta-cell sensitivity to incretins or changes in hepatic VLDL-palmitate export remains to be demonstrated.(26,27) However, metabolic dysfunction was less severe in C4 already before surgery and may reflect a state of metabolically healthy obesity.(28) Nonetheless, since prediabetes remission via weight loss is beneficial in terms of T2D risk reduction and potentially complications,(8) members of C4 with prediabetes may benefit from prediabetes remission despite the overall less severe metabolic dysfunction of the whole cluster. C5 benefitted most from BS in terms of improvement in insulin resistance, prediabetes remission and cardiovascular risk as reflected by the

in insulin resistance, prediabetes remission and cardiovascular risk as reflected by the

rFRS. Despite overall prediabetes remission rates were lower than expected after BS, individuals achieving prediabetes remission primarily improved insulin sensitivity in C6 and insulin secretion in C5. Thus, for members of C6 improving insulin sensitivity was sufficient to achieve prediabetes remission in light of the already high insulin secretion. Vice versa, improving insulin secretion appears to be a key mechanistic underpinning for those achieving remission in C5. Considering the slightly lower insulin secretion in C5 *vs* C6 at baseline, we cannot rule out that cluster definition may associate with this outcome.

Hepatic phenotypes of *Tübingen Clusters* previously assessed by ^{1}H MR-Spectroscopy are validated here for the first time via liver biopsies.(7) We observed that C5 indeed had the highest liver fat content, followed by C6, and reduced it into the near-normal range. The differential response to BS may result from stronger metabolic perturbances in C5 at baseline combined with the high effectiveness of BS in improving MASLD, shown to be associated with higher remission of T2D after BS.(29) This reduction in liver fat may be associated with an increase in insulin secretion as has been demonstrated previously.(30–32) Importantly, the non-invasive FLI shows a similar pattern as liver steatosis determined by liver biopsy. However, liver fibrosis assessed both by liver biopsy (Kleiner Liver Fibrosis Score) and non-invasively (APRI and NAFLD-FS) did not differ between clusters, reflecting the relatively low prevalence of liver fibrosis in this cohort. Additionally, non-invasive tests (NITs) for hepatic fibrosis in MASLD may not be suitable for adequately reflecting an elevated MASLD risk after BS (e.g. increasing FIB4 Scores in responders of C5 and C6).(24)

Weight loss was higher in individuals achieving prediabetes remission in all clusters, indicating that weight loss can mediate prediabetes remission which has previously been demonstrated for lifestyle intervention.(8) Although weight loss mediates

prediabetes remission at least in part in all clusters, mechanisms resulting in

prediabetes remission differ between clusters. Specifically, in C4, insulin resistance only improved marginally, while in C6, change in insulin sensitivity was a strong discriminator between response *vs* non-response. This was not the case in C5. Furthermore, neither non-responders nor responders from C4 augmented insulin secretion or beta-cell function significantly, while in C5 and C6, responders increased beta-cell function in particular. As the prediabetic state delineates a higher risk for T2D, the primary aim, beside weight loss, should be remission of prediabetes as one of the most effective ways to reduce T2D risk.(8,10) As shown here, prediabetes remission rates after BS differed between low-risk and high-risk clusters. This is yet surprising since individuals in C4 were younger compared to C5 and younger age has been associated with higher prediabetes remission rates after BS.(33) Overall remission rates of prediabetes were similar to those that have been observed after BS in individuals with overt T2D.(34) Since in prediabetes, metabolic derangements are not as severe as in T2D, a higher feasibility of prediabetes remission compared to T2D remission could be expected. However, similar remission rates suggest that prediabetes remission is as difficult to achieve as T2D remission. Similar to our findings, evidence on bariatric surgery-induced T2D remission in T2D sub-phenotypes showed that BS induced a lower remission rate in the low-risk mild obesity-related diabetes (MOD) compared to the high-risk cluster SIRD.(6) These findings indicate that other mechanisms apart from weight loss, which was similar between clusters in our study, might play a role in improving glucose regulation in C4. Specific characteristics of C4 that cannot or can only partly be improved by BS may be decisive for this. As C4 had near-normal HOMA-IR values at baseline, insulin resistance may not be the main driver of the prediabetic state in this cluster. Even though subjects in C4 had a slightly better beta-cell function at baseline compared to C5 and C6, still a higher proportion in

C4 did not manage to further improve beta-cell function as C5 and C6. Individuals with

severe insulin deficient diabetes (SIDD) have been shown to have the lowest diabetes remission rate when compared to MOD and SIRD.(6) In line with this, lacking the ability to increase beta-cell function could prevent individuals of C4 from returning to normal glucose regulation.(35) In previous studies by our group, C4 did not have a specific genetic risk of beta-cell dysfunction.(7) Since first-phase insulin secretion after BS is also orchestrated by release of glucagon-like peptide-1 (GLP-1), C4 might not promote or even might not be able to increase GLP-1 secretion after BS as strongly as the other clusters.(36) Therefore, further studies examining incretin responses after BS between sub-phenotypes are needed in order to investigate if tailored treatment (e.g. with incretin-based medication) might be a more effective treatment option in terms of prediabetes remission, specifically in C4.(37)

After short-term follow up, most individuals converted from high-risk clusters to low-risk clusters, while subjects from low-risk clusters as expected stayed in low-risk clusters. This cluster change is similar in both cohorts undergoing BS (ABOS and Rome). However, in the Control cohort most individuals change to high-risk clusters over time, imposing an increasing metabolic risk without surgical intervention. This is the first study to show that individuals change clusters after BS, which may reflect the reduced cardiometabolic risk upon BS assessed by a reduction of the rFRS. Interestingly, rFRS was reduced to similar levels between all three clusters although C5 had the highest rFRS before surgery. This is particularly important since participants of C5 were oldest, age being part of the BS-independent variables of the rFRS. Furthermore, both C5 and C6 increased kidney function represented by eGFR, which might reflect the kidney-protective effect of BS specifically in these clusters, which may not be the case for C4. After long-term follow up in individuals who did not undergo BS, a switch from high- to low-risk cluster is rare and many of the individuals

with former low risk turn to high-risk clusters. Thereby, reassignment to *Tübingen*

Clusters could help assess risk for T2D and complications after BS and may guide therapeutic approaches post-operatively.

Our study has some limitations. First, most study participants were White Europeans which may limit generalizability. Additionally, this is the first study to show cluster reassignment after BS, which could be affected by alterations in gastrointestinal glucose absorption and insulin secretion. Still, high-risk clusters had the strongest improvement of rFRS and eGFR, possibly reflecting the reduced risk represented by cluster change. Similarly, not all dynamic glucose regulation indices have been validated after BS. However, OGTT curves have been successfully compared to hyperinsulinemic-euglycemic clamp tests before and after BS in individuals without diabetes.(38) Thus, applied indices most likely reflect actual metabolic changes after BS. Finally, different prediabetes definitions, e.g. by WHO criteria with fasting glucose 110-125 mg/dL (6.1-6.9 mmol/L) and without HbA1c cutoff, or by 1-hour PG, may result in different prediabetes remission rates.(39,40)

In conclusion, our results support the relevance of this novel T2D risk classification in individuals with severe obesity and identified differing responses to surgery. Our analysis shows that subjects classified as high-risk C5 benefitted most from BS in terms of amelioration of insulin resistance, insulin secretion, prediabetes remission and risk cluster change. Low-risk C4 had the lowest prediabetes remission rate, suggesting that reaching weight loss targets may not be sufficient for achieving normal glucose regulation in this cluster. These findings may help to advance precision medicine approaches in BS.

Author Contributions

F.P. and A.L.B. conceptualized this work. R.J.v.S., F.P., L.S., V.R., A.S., P.B., H.V., G.M., E.C., C.G., O.V., K.Z., R.A., A.M., R.C., G.B., C.M., M.C., M.H., L.F., A.M., K.K., A.P., R.L., A.F., N.S., H.P., A.L.B., contributed substantially to the detailed conception and design of the study, the acquisition of data, or the data analysis and interpretation. L.S. performed the cluster classification with the help of R.W. and K.P. L.S., V.R., F.P. and R.J.v.S. drafted the manuscript. All authors contributed to the interpretation of data and critical revision of the manuscript. L.S., V.R. and F.P. had full access to all the data in the study. R.J.v.S. and F.P. are the guarantors of this work and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors read and approved the final manuscript.

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	4 (N=121)	5 (N=180)	6 (N=503)	p value
Age (years)				
Mean [SD]	37.5 [10.6]	41.1 [11.6]	36.9 [11.3]	<0.001
Median [IQR]	37.0 [15.0]	41.0 [17.3]	35.0 [18.0]	
Sex				
female	103 (85.1%)	152 (84.4%)	389 (77.3%)	0.0396
male	18 (14.9%)	28 (15.6%)	114 (22.7%)	
BMI (kg/m^2)				
Mean [SD]	42.7 [5.2]	46.4 [6.6]	47.5 [7.6]	<0.001
Median [IQR]	41.3 [4.5]	45.2 [7.8]	45.7 [9.0]	
Waist-to-hip ratio				
Mean [SD]	0.9 [0.1]	0.9 [0.1]	0.9 [0.1]	<0.001
Median [IQR]	0.9 [0.1]	0.9 [0.1]	0.9 [0.1]	
Glomerular filtration rate MDRD (mL/min)				
Mean [SD]	100.3 [21.6]	95.9 [20.2]	101.0 [21.3]	0.0195
Median [IQR]	97.2 [26.7]	95.3 [24.3]	98.3 [24.7]	
Liver fat content (%)				
Mean [SD]	14.8 [22.6]	28.8 [24.9]	21.6 [21.9]	<0.001
Median [IQR]	5.0 [19.0]	20.0 [35.0]	15.0 [26.0]	
Type of surgery				
Gastric banding	36 (29.8%)	34 (18.9%)	112 (22.3%)	0.0546
Gastric bypass	67 (55.4%)	105 (58.3%)	264 (52.5%)	
Sleeve gastrectomy	18 (14.9%)	41 (22.8%)	127 (25.2%)	
Triglycerides (mmol/L)				
Mean [SD]	1.1 [0.5]	1.9 [0.9]	1.3 [0.5]	<0.001
Median [IQR]	1.0 [0.6]	1.7 [0.9]	1.2 [0.6]	
HDL-C (mmol/L)				
Mean [SD]	1.3 [0.3]	1.1 [0.2]	1.2 [0.2]	<0.001
Median [IQR]	1.2 [0.4]	1.0 [0.3]	1.2 [0.3]	
HOMA-IR				
Mean [SD]	1.7 [0.6]	5.0 [3.0]	3.8 [2.0]	<0.001
Median [IQR]	1.7 [0.9]	4.4 [2.8]	3.3 [2.2]	
HOMA-B				
Mean [SD]	108.3 [113.2]	184.0 [112.1]	213.4 [118.7]	<0.001
Median [IQR]	98.7 [52.2]	155.2 [114.9]	186.5 [130.4]	
Glucose area under the curve				
Mean [SD]	780.1 [113.2]	1061.0 [107.6]	862.0 [109.4]	<0.001
Median [IQR]	784.6 [116.0]	1067.2 [132.5]	865.5 [139.9]	
Fasting plasma glucose (mmol/L)				
Mean [SD]	5.0 [0.4]	5.8 [0.6]	5.2 [0.5]	<0.001
Median [IQR]	5.1 [0.5]	5.8 [0.9]	5.2 [0.7]	
2-h plasma glucose (mmol/L)				
Mean [SD]	5.2 [1.1]	8.6 [1.2]	6.2 [1.2]	<0.001
Median [IQR]	5.1 [1.4]	8.5 [1.5]	6.2 [1.6]	
Fasting plasma insulin (mU/L)				

Mean [SD]	7.5 [2.6]	19.6 [11.0]	16.5 [8.1]	<0.001

Median [IQR]	7.6 [3.6]	17.3 [12.0]	14.7 [9.1]	
2-h plasma insulin (mU/L)				
Mean [SD]	21.9 [14.2]	133.0 [104.0]	74.9 [62.1]	<0.001
Median [IQR]	18.2 [16.0]	101.2 [104.5]	62.2 [57.9]	
Fasting c-peptide (ng/mL)				
Mean [SD]	2.9 [1.9]	4.0 [1.1]	3.7 [1.1]	<0.001
Median [IQR]	2.7 [1.0]	3.8 [1.3]	3.5 [1.3]	
2-h c-peptide (ng/mL)				
Mean [SD]	7.3 [4.7]	12.9 [3.9]	10.2 [3.2]	<0.001
Median [IQR]	6.5 [3.0]	12.7 [4.1]	10.2 [4.2]	
HbA1c (%)				
Mean [SD]	5.5 [0.4]	5.7 [0.4]	5.6 [0.4]	<0.001
Median [IQR]	5.5 [0.5]	5.8 [0.5]	5.6 [0.5]	

Table 1: Baseline characteristics of ABOS (Lille) cohort
Data are median (IQR); mean (SD) or n (%). HOMA-IR=Homeostatic Model Assessment for Insulin Resistance. HOMA-B=Homeostasic Model Assessment of beta-Cell Function. HDL-C=High Density Lipoprotein Cholesterol. MDRD=Modification of Diet in Renal Disease.

Figure legends:

Figure 1: *Tübingen Clusters* distribution at baseline and change of cluster assignment over time.
(A) In ABOS (Lille) cohort. (B) In Rome cohort. (C) In Control Cohort (Tübingen).

Figure 2: Weight and glucose regulation trajectories in ABOS (Lille) cohort.
Percentage change of the following parameters (A) Weight loss. (B) $AUC_{Glucose\ 0\text{-}120\ min}$ (Glucose Area Under the Curve). (C) HOMA-IR=Homeostatic Model Assessment for Insulin Resistance. (D) Matsuda Index. (E) Disposition Index. (F) AUC=area under the curve. $AUC_{C\text{-}Peptide\ 0\text{-}30min}$ divided by $AUC_{Glucose\ 0\text{-}30min}$. (G-J) Plasma glucose and insulin trajectories over oGTT at baseline (G-H) and after 1 year (I-J). Data represents mean ± CI. *p < 0.05, **p <0.01 ***p <0.001, color of asterisks indicates compared cluster (G-J). Analysis was performed using two-way anova or Wilcoxon signed-rank test, as applicable.

Figure 3:
Glucose regulation trajectories, prediabetes remission and glucose regulation indices by response in ABOS (Lille) cohort.
(A) Glucose regulation status at baseline and 1 and 2 years after surgery in cluster 4. (B) In cluster 5. (C) in cluster 6. (D) Prediabetes remission after 1 year. (E) Prediabetes remission after 2 years. (F) Weight. (G) HOMA-IR=Homeostatic Model Assessment for Insulin Resistance. (H) Matsuda Index. (I) HOMA-B=Homeostasic Model Assessment of beta-Cell Function. (J) Disposition Index. (K) AUC=area under the curve. $AUC_{C\text{-}Peptide\ 0\text{-}30min}$ divided by $AUC_{Glucose\ 0\text{-}30min}$. This multivariable mixed linear model included age, sex, type of surgery, timepoint, cluster, and the interaction between cluster and timepoint as main effects. Data represents mean ± CI. *p < 0.05, **p <0.01 ***p <0.001, color of asterisks indicates compared cluster.