Different effects of lifestyle intervention in high- and low-risk prediabetes

Results of the randomized controlled Prediabetes Lifestyle Intervention Study (PLIS)

Running title: Risk-stratified lifestyle intervention in prediabetes

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Abstract

Lifestyle intervention (LI) can prevent type 2 diabetes, but response to LI varies depending on risk subphenotypes. We tested if prediabetic individuals with low risk benefit from conventional LI and individuals with high risk benefit from an intensification of LI in a multi-center randomized controlled intervention over 12 months with 2 years follow up. 1105 prediabetic individuals based on ADA glucose criteria were stratified into a high- and low-risk phenotype, based on previously described thresholds of insulin secretion, insulin sensitivity and liver fat content. Low-risk individuals were randomly assigned to conventional LI according to the DPP protocol or control (1:1), high-risk individuals to conventional or intensified LI with doubling of required exercise (1:1). A total of 908 (82%) participants completed the study. In high-risk individuals, the difference between conventional and intensified LI in post-challenge glucose change was -0.29 mmol/l [CI:-0.54;-0.04], p=0.025. Liver fat (-1.34 percentage points [CI:-2.17;-0.50], p=0.002) and cardiovascular risk (-1.82[CI:-3.13-0.50],p=0.007) underwent larger reductions with intensified than with conventional LI. During a follow up of 3 years, intensified compared to conventional LI had a higher probability to normalize glucose tolerance (p=0.008). In conclusion, it is possible in high-risk individuals with prediabetes to improve glycemic and cardiometabolic outcomes by intensification of LI. Individualized, risk-phenotype-based LI may be beneficial for the prevention of diabetes.

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Keywords:

stratified randomization, randomized clinical multi-center trial, lifestyle intervention, diabetes, prediabetes

Lifestyle modification is the principal procedure for type 2 diabetes prevention in individuals with prediabetes. During the last two decades, multiple studies have shown that lifestyle intervention (LI) is effective in preventing diabetes. Several prospective randomized studies (1-4) have demonstrated that diabetes risk can be reduced by modifying diet and physical exercise. Such approaches yield relative diabetes risk reductions between 15% and 70% within 1 to 6 years of follow-up (5). Recent meta-analyses of randomized trials reported mean risk ratios of 0.35 (6), 0.57 (7) and 0.61 (8) when comparing LI to usual care. This points to a robust benefit of LI for the prevention of type 2 diabetes, which is sustainable and extends beyond the duration of the intervention (4,9,10).

Nevertheless, there is a pressing need for making LI more efficient for diabetes prevention because a considerable proportion of participants in LI trials do not benefit from the intervention. They are often referred to as "non-responders" (11,12). For example, every fifth patient of the LI group in the Diabetes Prevention Programme (DPP) developed type 2 diabetes within four years (2). An alternative definition of non-response is the inability to regress from prediabetes to a normal glucose regulation during a LI program (11). In the DPP, only ~40% of participants accomplished regression to normal glucose regulation (11), i.e. 60% were LI non-responders. Furthermore, there is the important question whether lifestyle intervention is necessary in all individuals with prediabetes (13). There are individuals with prediabetes who do not progress to diabetes during 11 years of follow up even without intervention (14). In such individuals with "intermediate hyperglycemia", lifestyle intervention with the sole purpose to lower blood glucose might be of less importance. Both observations of non-response to LI and non-progression to diabetes highlight the need for risk stratified intervention strategies in individuals with prediabetes.

The fundamental question is which phenotypes determine the risk for diabetes and especially the response and non-response to LI. A recent *post hoc* analysis of the DPP showed that response varies based on diabetes risk (15), suggesting an adaption of LI on individual risk.

In a retrospective analysis of the Tuebingen Lifestyle Intervention Program (TULIP) study, we identified a high-risk phenotype associated with higher probability of short-term (16) and long-term non-response (12) to LI. This phenotype represents beta cell dysfunction and/or insulin resistant non-alcoholic fatty liver disease (NAFLD), which is also associated with increased cardiometabolic risk (17). Similar phenotypes have been identified by cluster analysis in type 2 diabetes or prediabetes patients (18,19). These approaches show that risk-stratification can identify severe disease courses and increased risk for diabetes-related complications both in populations prior to diabetes onset and with diabetes.

Therefore, it is also crucial to improve the efficiency and effectiveness of LI programs in high-risk participants to overcome non-response to preventive interventions. Unnecessary overtreatment can be avoided by identification of low-risk individuals who do not need treatment. We had designed a prospective risk-stratified randomized controlled multi-center LI study. Within the PLIS study, we performed 2 randomized controlled trials, one in the high risk individuals to answer the questions (i) an non-response in high-risk individuals with prediabetes be overcome by intensification of LI? and the second one in low risk individuals to answer the question (ii) Is lifestyle intervention effective in low-risk individuals with prediabetes? The primary hypothesis is that individuals with prediabetes who have high risk for a failure to restore normal glucose regulation with conventional LI will benefit from an intensification of the LI.

Research Design and Methods

Study design

The prediabetes lifestyle intervention study (PLIS) (ClinicalTrials.gov Identifier: NCT01947595) is a stratified randomized multi-center trial involving eight study sites in university hospitals in Germany (Appendix Table 1). Prediabetes was diagnosed from fasting and 2 hour post-challenge glucose (2hPG) levels after a standardized oral glucose tolerance test (OGTT), according to the criteria of the American Diabetes Association (20). HbA1c was not used as a definition for prediabetes. Screening procedures also involved measurement of liver fat content, insulin sensitivity and insulin secretion. Based on previously established cut-off levels (16), these variables were used for risk stratification. High risk participants (HR) were characterized by a reduced insulin secretion (Disposition index, DI) and/or insulin resistance (low insulin sensitivity index, ISI) and elevated liver fat content. Cut off levels for risk stratification (HR vs LR) were <760 AU (DI, reduced insulin secretion), <9.2 AU (ISI, reduced insulin sensitivity) and >5.56% (liver fat content MRT) (16). For calculation of indices see Supplementary Appendix. Low-risk participants (LR) were randomized to receive no lifestyle intervention (control group, LR-CTRL) or a conventional (LR-CONV) lifestyle intervention. Participants with high-risk (HR) were randomized to receive either a conventional lifestyle intervention (HR-CONV) or an intensive lifestyle intervention (HR-INT). Randomisation was performed using a computer-based block-randomisation at the center of Tübingen by a study supervisor. For this, a self-devised randomiser with a permuted block randomization with a block size of 30 was used. At each study site, the study personnel was blinded, except for the principal investigator and the personnel performing the actual lifestyle counselling. Participants were enrolled between 2012 and 2016. The study protocol was approved by all local ethics committees of the participating institutions. This study has been reporting in line with the

CONSORT guidelines and the completed checklist is in the Supplementary Appendix. The detailed study protocol is available online.

Study outcomes

The primary outcome measure 2hPG was assessed by an OGTT after 12 months, an intermediate OGTT was performed after 6 months. Secondary outcome measures were liver fat content, insulin sensitivity and secretion and cardiovascular risk. Insulin sensitivity was calculated using glucose and insulin levels obtained during the OGTT with the equation of Matsuda and DeFronzo (ISI) (21). Insulin secretion was calculated with the insulinogenic index (IGI) (22). To obtain insulin secretion capacity adapted for the actual insulin sensitivity, the disposition index (ISI×IGI) was used. Cardiovascular risk was assessed with the Framingham risk score which was calculated using the equation provided by D'Agostino et al. (23), with participants having concomitant IFG and IGT treated for this calculation as participants with diabetes.

Tertiary outcomes measures were adherence to the lifestyle intervention measures rated by a continuous score reaching from 0 to 5 (see below).

Participants

Individuals participated in a screening OGTT if they had clinically suspected prediabetes, or at least 50 points in the German Diabetes Risk assessment battery (24). Basic inclusion criteria comprised age between 18 and 75 years and a BMI < 45 kg/m^2 and diagnosis of impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT). Exclusion criteria are listed in Appendix Table 2. All participants provided written informed consent.

Intervention

The duration of the LI was 12 months. In both the conventional and the intensified treatment groups, the lifestyle intervention was aimed at reaching a body weight reduction of 5% in participants with a body mass index (BMI) > 25 kg/m^2 by reducing fat intake to less than 30% of total energy intake, reducing saturated fat intake to less than 10% of total energy intake, and increasing fiber intake to more than 15 g per 1000 kcal of total energy intake. Participants of the conventional intervention group received eight LI sessions in total over 1 year. They were advised to perform 3 hours of exercise weekly. Participants of the intensified LI group received 16 coaching sessions in total over 1 year with advice to exercise 6 hours weekly. The duration of the one-to-one coaching sessions was 30-60 minutes. They included dietary counselling based on diet protocols completed by the participants on four consecutive days. Furthermore, exercise counselling was performed on the basis of data from accelerometers, also enabling the assessment of accomplishing exercise goals. During each visit, lifestyle advisors graded adherence to the 5 goals of intervention (3 diet, 1 exercise goal, 1 weight reduction goal based on diet and exercise protocols and if a weight reduction <5% was reached). After one year of intervention, a total score was computed for all participants, with each of the 5 goals rated as 1 when achieved and 0 if not, and aggregated. This sum score therefore ranges from 0 (none of the goals achieved) to 5 (all 5 goals achieved). All dieticians/lifestyle advisors were trained using the same curriculum (10h) by a team from the primary site before starting recruitment. Refresher courses and face to face meetings between advisors were organized at least yearly between the study centers in workshops to ensure team building and harmonized counselling across all study sites involved. The LI was based on previously published established curricula (Diabetes Prevention Study (DPS), DPS, TULIP (1,2,12)).

Participants of the control group only received a single 30 minutes one-to-one consultation with a dietician at baseline.

Oral glucose tolerance test and analytical procedures

OGTTs were performed at 8:00 after an overnight fast. Participants ingested 75 g glucose (Accu-Check Dextro O.G.T., Roche). Blood samples were obtained at fasting, 30, 60, 90 and 120 minutes via an indwelling venous catheter. Blood samples were immediately put on ice and frozen at -80°C.

Glucose levels were measured locally at the study sites in certified laboratories using glucose oxidase method. Plasma insulin was measured centrally in the laboratory of the Tübingen University Hospital with a commercial chemiluminescence assay on an ADVIA Centaur XP (Siemens Healthineers). Clinical chemistry parameters (alanine and aspartate aminotransferase, gamma-glutamyltransferase, HbA1c, total, HDL, and LDL cholesterol and triacylglycerol) were determined under quality-ensured conditions in the local routine diagnostic laboratories (see appendix table 1), all certified by the German accreditation council (DAkkS). Internal and external quality assessments and proficiency testing was performed at all times of the study in each of these diagnostic laboratories. In the DZD central clinical chemistry lab at the University Hospital Tübingen the above mentioned analytes were measured on the ADVIA XPT Clinical Chemistry system (Siemens Healthineers, Eschborn, Germany) and Tosoh G8 HPLC analyzer (Tosoh Bioscience, Griesheim, Germany).

Magnetic resonance imaging and spectroscopy

Liver fat content was determined by localized proton magnetic resonance spectroscopy (^{1}H-MRS) using stimulated echo acquisition mode in the posterior hepatic segment 7 (25). Liver fat content was determined by the ratio of signal integrals of fat (methylen+methyl signal) and total signal (water+fat), expressed in %. ^{1}H-MRS was not available in one center. Here, liver fat content was quantified by a chemical-shift selective imaging technique generating fat and water selective images (26). Liver fat content was determined from a manually drawn region of

interest in segment 7, performed separately on the water selective and the fat selective image. Similar to the ^{1}H-MRS method, liver fat content was calculated as fat/(water+fat)*100 correcting for relaxation effects in order to make the imaging approach comparable to MRS. Both methodological approaches enable an accurate and comparable quantification of liver fat (27). For a small proportion of participants who were unable to undergo magnetic resonance studies or when magnetic resonance studies were not available, hepatic steatosis was assessed using ultrasound criteria to detect fatty liver as previously described (28) to allow risk stratification.

Statistical Analysis

Given a type 1 error probability of 0.05 (alpha) and a type 2 error probability of 0.2 (beta), the study was designed to be powered to detect a difference of 0.44 mmol/l in post-challenge glucose in a study population of 200 per intervention group. A complete cases approach was used for all analyses. We performed a sensitivity analysis after imputation of the missing variables using multivariable imputation performed on a wide-dataset encompassing basic variables (sex, age, BMI, waist circumference, education, study center), and glycemic variables (glucose during OGTT, AUC glucose, HbA1c), variables on insulin secretion and sensitivity (ISI and IGI), disposition index as well as liver fat content at baseline and follow-up at 12 months. The imputation was performed using the MICE package in R using default settings (predictive mean matching as default algorithm, 5 iterations) and passive imputation for derived variables (disposition indexes).

The primary and secondary endpoints were analysed by general linear models. For example, as primary end-point, post-challenge glucose at the end of the intervention was evaluated with ANCOVA with the model terms intervention, baseline post-challenge glucose and study center as fixed effects. For each other outcome at the follow-up visit, we used the outcome at baseline visit, intervention and study centre as model terms. Results from general linear models are

provided as beta estimates in the results section and, for the specific intervention groups, as least-square means with 95% CI (Table 2). All other tables show means and standard deviation. In addition, we have conducted post-hoc tests using alternative insulin sensitivity and secretion variables to predict the primary outcome. The prediction power was very similar to our current approach; therefore, we think that the kind of indices estimating insulin sensitivity and secretion do not critically influence our results. Post hoc power analyses showed achieved statistical powers of 0.26 for the low-risk and 0.64 for the high risk trials.

Due to follow-up visits at pre-specified time-points, we considered our data as interval-censored for the computation of the regression to normal glucose tolerance. We approximated the baseline hazard with an exponential distribution and used this in full parametric proportional odds survival models in both risk groups.

All statistical analyses were performed in R (Version 3.4) (29). GLMs were fitted with the lm function in R using default settings, the survival models were fitted with the icenReg package.

Data and Resource Availability

Information is listed in the Appendix

Results

Study participants

Out of 2561 individuals with increased risk for diabetes, a total of 1160 individuals were identified as eligible, agreed to participate and underwent risk stratification into a low-risk group (LR) and a high-risk group (HR). A total of 1105 individuals were subsequently randomized into the four study groups and received allocated intervention. Details can be seen in Figure 1.

After one year, 908 individuals (82%) completed the study, and outcome data for the primary endpoint (complete glucose data from OGTT) were obtained. Among these, high-risk subjects were significantly older and had higher BMI. They also differed in all major metabolic traits such as glucose and lipid levels, insulin sensitivity and insulin secretion (see Table 1). The randomization procedure resulted in balanced demographic and clinical characteristics between LR-CTRL and LR-CONV as well as between HR-CONV and HR-INT (see Appendix Table 4). Non-completers did not differ from completers regarding the allocation to risk groups and intervention arms. Non-completers were significantly more often female, younger and had higher BMI (see Appendix Table 5).

Primary outcome: post-challenge glucose

Post-challenge glucose levels decreased in all study groups (See Table 2).

In high-risk subjects, the mean difference estimate between conventional and intensified LI of the change of post-challenge glucose levels from baseline to 1 year follow-up was -0.29 mmol/l [CI: -0.54;-0.04], p=0.025, adjusted for baseline and center (see Figure 2A). For the least-square means of changes from baseline to follow up in each intervention group, see Table 2. In low-risk subjects, the change in 2hPG was not significantly different between the LR-CTRL and

LR-CONV groups (mean difference estimate, 0.19 mmol/l [CI: -0.22;0.60], p=0.4, see Figure 2A).

Regression to normal glucose tolerance during long-term follow up

We extended our study to perform follow-up visits after the LI, including an OGTT after 1 and 2 years. During this total observation period of 3 years, intensive LI led to a cumulative higher conversion rate to normal glucose tolerance in high risk individuals compared to conventional LI (HR 1.57 [CI: 1.17;2.1], p=0.003, parametric proportional odds survival model using an exponential baseline risk distribution, Figure 3). In low-risk individuals, participants receiving conventional lifestyle had a higher chance of conversion to normal glucose tolerance compared to controls during 3 years of follow-up (HR 2.02 [CI: 1.18;3.43], p=0.01).

Secondary outcomes (BMI, insulin sensitivity and secretion, liver fat content, cardiometabolic risk) and sensitivity analysis

In high-risk subjects, the mean difference estimate between intensified and conventional LI for the change in liver fat content was -1.34 % [CI: -2.17;-0.5], p=0.002. For cardiometabolic risk score, this difference was -1.82 [CI: -3.13;-0.5], p=0.007, for insulin sensitivity 0.64 AU [CI: 0.13;1.15], p=0.01 and for BMI -0.47 kg/m² [CI: -0.74;-0.2], p<0.001 in the HR-INT group compared to the HR-CONV group after 1 year of LI (see Table 2 and Figure 2). The change in insulin secretion was similar in the HR-CONV and HR-INT groups.

In low-risk subjects, there were no statistically significant differences between conventional LI and controls, with the exception of BMI and fasting glucose (see table 2).

As sensitivity analysis, we imputed all missing variables for the baseline and follow-up visit and computed the main outcomes in the imputed dataset. The significance levels of the results

were consistent with the analysis of complete cases (see Appendix Table 3), but effect sizes tended to be higher.

Adherence

The main study outcome 2hPG was associated with the aggregate percentage of completed lifestyle goals (both modelled as continuous variable, analyzed in a baseline-adjusted linear model, β±SE=-0.09±0.03, p=0.001). In a baseline-adjusted multivariable model comprising all specific lifestyle goals, only achievement of weight reduction (β±SE=-0.18±0.03, p<0.001) and exercise goals (β±SE=-0.07±0.03, p=0.02) were independently associated with 2hPG. In addition, the number of completed visits during the study was also positively associated with 2hPG in a model adjusted for baseline post-challenge glucose and intervention (β=-0.23±0.1, p=0.02).

The aggregate percentage of completed lifestyle goals was higher in the LR-CONV than in the HR-CONV (mean±SD 45±3% vs 38±1%, p=0.03, Wilcoxon-test, Appendix Figure 2). In high-risk subjects, the aggregate percentage of completed lifestyle goals was similar in the HR-CONV (38±1%) compared to the HR-INT group (41±1%, p=0.5, Wilcoxon-test). When investigating the specific goals within the high-risk groups, more individuals reached exercise goals in the HR-CONV group, the weight goals were achieved by more individuals in the HR-INT group (both p<0.001, chi-squared test).

Safety and Adverse events

There were 0.88 adverse events per patient year. After adjusting for the number of visits and for centers, the frequency of adverse events was not different between all 4 risk groups (all p>0.5, Poisson regression). No severe adverse events were recorded during the trial.

Discussion

In the present multicenter, risk-stratified, randomized, controlled lifestyle intervention trial, our primary aim was to test whether individuals with prediabetes and a high-risk phenotype with impaired insulin secretion and/or insulin resistant fatty liver benefit from an intensification of conventional LI. The PLIS study showed that in this population at high risk for diabetes, intensification of LI by increasing counselling frequency and weekly physical exercise indeed yielded a superior improvement of the primary outcome, i.e. postprandial glucose metabolism after one year of LI. In addition, these participants undergoing intensive LI were also more probable to reduce secondary outcomes such as liver fat content and cardiometabolic risk. In a second randomized trial within the PLIS study, we additionally tested if individuals with a low-risk phenotype benefit from conventional LI compared to no LI. In these participants, we detected no difference of the primary outcome postprandial glucose metabolism. However, a smaller sample size in the low-risk stratum resulting in a low power might have precluded detection of smaller differences.

The stratification between the low risk and high-risk phenotype is defined by pathophysiological features of type 2 diabetes and has been described previously (12,16,17). The determinants of this phenotype, impaired insulin secretion and insulin resistance, are the main pathomechanisms for the development of type 2 diabetes (30-34).

Our data indicate that conventional lifestyle interventions, as were applied in the DPS (1) and DPP (2), can be successfully intensified. This argues for a dose-effect relationship in LI. By applying the intensified intervention, beneficial effects on body mass index, insulin sensitivity, and liver fat content were more pronounced. In contrast, intensified LI did not improve insulin secretion capacity compared to conventional LI (table 2 and appendix figure 1). Therefore, the superior effect of intensified LI on post-challenge glucose seems mainly due to reduced liver fat content and improved insulin sensitivity. The changes of liver fat content and insulin

sensitivity were significantly associated with improvement of glucose tolerance, independent of change in body weight in the high-risk population (β=0.045, p=0.02 and β=-0.12, p<0.0001, respectively). The importance of improved insulin sensitivity in successful LI is consistent with findings from the DPP and DPS trials (35,36), whereas the data about the role of liver fat reduction is new.

The intensified and conventional intervention in PLIS differed in regard of exercise volume and the amount of counselling sessions. Of note, the number of completed visits and the accomplishment of the weight reduction goal were significantly associated with the reduction of 2hPG during one year of intervention in all treatment groups. This suggests that the amount of counselling and either more motivation or more guidance from lifestyle advisors underlies the higher efficacy of the intensive treatment group. Qualified lifestyle counsellors and an adequate counselling frequency should be key factors in LI planning. One additional important aspect are the perceptions and quality of life of participants taking part in the different lifestyle interventions. Quality of life during long term follow up and the feasibility of such lifestyle intervention in a real-world situation is being analysed in a separate project.

One feature of the PLIS study was that we additionally tested the effect of conventional LI in the group with low risk for LI non-response by comparing conventional LI with a group who did not receive LI. No difference was found for the primary endpoint 2hPG between those groups. However, based on the limited statistical power reached in the low risk group due to the smaller sample size of this group, we cannot exclude a false negative finding with acceptable confidence.

Several studies have shown that translating the promising results of controlled lifestyle interventions into a real-world scenario is hardly possible (37,38). Risk stratification during screening and subsequent allocation of resources to individuals who are at marked risk may improve outcomes and cost-effectiveness. For example, in individuals with type 2 diabetes, no advantage of a LI on cardiovascular disease mortality and morbidity was shown in the Look

AHEAD trial (39). However, a post-hoc analysis has recently identified a subgroup that benefited from the LI. Individuals with well controlled diabetes (low risk) and poor self-reported general health did not benefit from the intervention (40). A screen and treat policy for the prevention of type 2 diabetes will be effective when it is possible to prospectively identify individuals at high-risk while excluding those at low risk (41). The current study provides a proof of concept for this approach.

Importantly, the beneficial effects of intensified LI reach beyond glucose control. The present study is the largest multicenter randomized LI trial measuring liver fat content with a highly reliable technique of magnetic resonance spectroscopy. Hepatic steatosis is present in 25% of the adult population in the United States, and is associated with diabetes, cardiovascular disease, steatohepatitis and liver cancer (42). In high-risk individuals, we achieved a relative liver fat reduction of 37% with intensified intervention, whereas conventional intervention only resulted in a relative reduction of 24%. Individuals who participated in the intensified intervention group achieved reduced liver fat content of 6.6±0.5% compared with 8.3±0.5% in those undergoing conventional intervention. This means that liver fat content was close to the normal threshold of 5.6% after the intensified intervention which therefore implies a clinically relevant effect and should be a target for future approaches to diabetes prevention.

Furthermore, the cardiovascular risk diminished in the participants of the high-risk stratum with a near doubling of risk reduction after intensified intervention, compared to conventional LI (see Table 2 and Figure 2).

Limitations of our study include the relative short LI duration of 12 months and a non-completer rate of 18% after one year. The latter is, however, well in the range of other LI studies with rates between 5% and 28% (5). A potential further limitation is the heterogeneity of lifestyle counselling throughout different study centers which could have been reduced by more frequent meetings and interactions between study sites. Furthermore, the design of the present study did not include an intensified intervention in the low-risk group, therefore we were unable to test

whether the intensified LI would work in low risk individuals. Therefore, it may be possible that the level of physical activity was not sufficient to improve outcome in this group. In addition, there was no control group with no intervention in the high-risk group. Moreover, the high risk and low risk group were unbalanced with more individuals stratified to the high-risk group (78%). Thus, one of the predefined questions "is lifestyle intervention effective in low-risk individuals with prediabetes" cannot be answered with high confidence in the current study due to a low statistical power reached in this group.

To our knowledge, this is the first multicenter study that prospectively tested different intensities of lifestyle intervention in a risk-stratified manner. PLIS confirms the existence of a high-risk phenotype for non-response to LI in individuals with prediabetes. This non-response can be partially compensated with intensified LI such that a higher percentage of high-risk individuals improve glucose metabolism, decrease liver fat content and cardiovascular risk. Finally, conventional lifestyle intervention with the aim of improving glucose tolerance in individuals with prediabetes and low risk might also be important. Future studies are needed to explicitly investigate this question in low risk persons. Nonetheless, screen and treat approaches in the prevention of type 2 diabetes should include risk stratification and individualized interventions.

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Duality of interest

No potential conflicts of interest relevant to this article are reported

Authors Contributions

AF, NS, and HUH conceived the study. AF, RW and ML analysed the data. AF and RW wrote the manuscript. AF, RW, MH, KK, PPN, AFP, MS, AB, HH, JS, AL, KW, SB, MR, NS, HUH contributed to interpretation of the data and edited the MS. All other authors contributed to data acquisition and approved the final version of the manuscript. AF is guarantor and attests all listed authors meet authorship criteria and no others meeting the criteria have been omitted. The lead author (AF) affirms that the manuscript is an honest, accurate, and transparent account of the study being reported and that no important aspects of the study have been omitted.

References

1. Tuomilehto J, Lindström J, Eriksson JG, Valle TT, Hämäläinen H, Ilanne-Parikka P, Keinänen-Kiukaanniemi S, Laakso M, Louheranta A, Rastas M, Salminen V, Uusitupa M; Finnish Diabetes Prevention Study Group. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. N Engl J Med 2001;344:1343-1350

2. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Nathan DM; Diabetes Prevention Program Research Group. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med 2002;346:393-403

3. Ramachandran A, Snehalatha C, Mary S, Mukesh B, Bhaskar AD, Vijay V; Indian Diabetes Prevention Programme (IDPP). The Indian Diabetes Prevention Programme shows that lifestyle modification and metformin prevent type 2 diabetes in Asian Indian subjects with impaired glucose tolerance (IDPP-1). Diabetologia 2006;49:289-297

4. Li G, Zhang P, Wang J, Gregg EW, Yang W, Gong Q, Li H, Li H, Jiang Y, An Y, Shuai Y, Zhang B, Zhang J, Thompson TJ, Gerzoff RB, Roglic G, Hu Y, Bennett PH. The long-term effect of lifestyle interventions to prevent diabetes in the China Da Qing Diabetes Prevention Study: a 20-year follow-up study. Lancet 2008;371:1783-1789

5. Yoon U, Kwok LL, Magkidis A. Efficacy of lifestyle interventions in reducing diabetes incidence in patients with impaired glucose tolerance: a systematic review of randomized controlled trials. Metabolism 2013;62:303-314

6. Schellenberg ES, Dryden DM, Vandermeer B, Ha C, Korownyk C. Lifestyle interventions for patients with and at risk for type 2 diabetes: a systematic review and meta-analysis. Ann Intern Med 2013;159:543-551

7. Hemmingsen B, Gimenez-Perez G, Mauricio D, Roqué I Figuls M, Metzendorf MI, Richter B. Diet, physical activity or both for prevention or delay of type 2 diabetes mellitus and its associated complications in people at increased risk of developing type 2 diabetes mellitus. Cochrane Database Syst Rev 2017;12:Cd003054

8. Haw JS, Galaviz KI, Straus AN, Kowalski AJ, Magee MJ, Weber MB, Wei J, Narayan KMV, Ali MK. Long-term Sustainability of Diabetes Prevention Approaches: A Systematic Review and Meta-analysis of Randomized Clinical Trials. JAMA Intern Med 2017;177:1808-1817

9. Diabetes Prevention Program Research Group, Knowler WC, Fowler SE, Hamman RF, Christophi CA, Hoffman HJ, Brenneman AT, Brown-Friday JO, Goldberg R, Venditti E, Nathan DM. 10-year follow-up of diabetes incidence and weight loss in the Diabetes Prevention Program Outcomes Study. Lancet 2009;374:1677-1686

10. Lindström J, Ilanne-Parikka P, Peltonen M, Aunola S, Eriksson JG, Hemiö K, Hämäläinen H, Härkönen P, Keinänen-Kiukaanniemi S, Laakso M, Louheranta A, Mannelin M, Paturi M, Sundvall J, Valle TT, Uusitupa M, Tuomilehto J; Finnish Diabetes Prevention Study Group. Sustained reduction in the incidence of type 2 diabetes by lifestyle intervention: follow-up of the Finnish Diabetes Prevention Study. Lancet 2006;368:1673-1679

11. Perreault L, Kahn SE, Christophi CA, Knowler WC, Hamman RF; Diabetes Prevention Program Research Group. Regression from pre-diabetes to normal glucose regulation in the diabetes prevention program. Diabetes Care 2009;32:1583-1588

12. Schmid V, Wagner R, Sailer C, Fritsche L, Kantartzis K, Peter A, Heni M, Häring HU, Stefan N, Fritsche A. Non-alcoholic fatty liver disease and impaired proinsulin conversion as newly identified predictors of the long-term non-response to a lifestyle intervention for diabetes prevention: results from the TULIP study. Diabetologia 2017;60:2341-2351

13. Piller C. Dubious diagnosis. Science 2019;363:1026-1031

14. Richter B, Hemmingsen B, Metzendorf MI, Takwoingi Y. Development of type 2 diabetes mellitus in people with intermediate hyperglycaemia. Cochrane Database Syst Rev 2018;10:Cd012661

15. Sussman JB, Kent DM, Nelson JP, Hayward RA. Improving diabetes prevention with benefit based tailored treatment: risk based reanalysis of Diabetes Prevention Program. BMJ 2015;350:h454

16. Stefan N, Staiger H, Wagner R, Machann J, Schick F, Häring HU, Fritsche A. A high-risk phenotype associates with reduced improvement in glycaemia during a lifestyle intervention in prediabetes. Diabetologia 2015;58:2877-2884

17. Stefan N, Fritsche A, Schick F, Häring HU. Phenotypes of prediabetes and stratification of cardiometabolic risk. Lancet Diabetes Endocrinol 2016;4:789-798

18. Ahlqvist E, Storm P, Käräjämäki A, Martinell M, Dorkhan M, Carlsson A, Vikman P, Prasad RB, Aly DM, Almgren P, Wessman Y, Shaat N, Spégel P, Mulder H, Lindholm E, Melander O, Hansson O, Malmqvist U, Lernmark Å, Lahti K, Forsén T, Tuomi T, Rosengren AH, Groop L. Novel subgroups of adult-onset diabetes and their association with outcomes: a data-driven cluster analysis of six variables. Lancet Diabetes Endocrinol 2018;6:361-369

19. Wagner R, Heni M, Tabák AG, Machann J, Schick F, Randrianarisoa E, Hrabě de Angelis M, Birkenfeld AL, Stefan N, Peter A, Häring HU, Fritsche A. Pathophysiology-based subphenotyping of individuals at elevated risk for type 2 diabetes. Nat Med. 2021 Jan;27(1):49-57.

20. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2021. Diabetes Care 2021;44:S15-S33.

21. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care 1999;22:1462-1470

22. Seltzer HS, Allen EW, Herron AL Jr, Brennan MT. Insulin secretion in response to glycemic stimulus: relation of delayed initial release to carbohydrate intolerance in mild diabetes mellitus. J Clin Invest 1967;46:323-335

23. D'Agostino RB Sr, Vasan RS, Pencina MJ, Wolf PA, Cobain M, Massaro JM, Kannel WB. General cardiovascular risk profile for use in primary care: the Framingham Heart Study. Circulation 2008;117:743-753

24. Schulze MB, Hoffmann K, Boeing H, Linseisen J, Rohrmann S, Möhlig M, Pfeiffer AF, Spranger J, Thamer C, Häring HU, Fritsche A, Joost HG. An accurate risk score based on anthropometric, dietary, and lifestyle factors to predict the development of type 2 diabetes. Diabetes Care 2007;30:510-515

25. Machann J, Thamer C, Schnoedt B, Stefan N, Haring HU, Claussen CD, Fritsche A, Schick F. Hepatic lipid accumulation in healthy subjects: a comparative study using spectral fat-selective MRI and volume-localized 1H-MR spectroscopy. Magn Reson Med 2006;55:913-917

26. Liu CY, McKenzie CA, Yu H, Brittain JH, Reeder SB. Fat quantification with IDEAL gradient echo imaging: correction of bias from T(1) and noise. Magn Reson Med 2007;58:354-364

27. Kim H, Taksali SE, Dufour S, Befroy D, Goodman TR, Petersen KF, Shulman GI, Caprio S, Constable RT. Comparative MR study of hepatic fat quantification using single-voxel proton spectroscopy, two-point dixon and three-point IDEAL. Magn Reson Med. 2008;59:521-527

28 . Schwenzer NF, Springer F, Schraml C, Stefan N, Machann J, Schick F. Non-invasive assessment and quantification of liver steatosis by ultrasound, computed tomography and magnetic resonance. J Hepatol. 2009;51:433-445

29. R: A Language and Environment for Statistical Computing: R Foundation for Statistical Computing, 2015 https://www.R-project.org.

30. Weyer C, Bogardus C, Mott DM, Pratley RE.. The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus. J Clin Invest 1999;104:787-794

31. Pratley RE, Weyer C. The role of impaired early insulin secretion in the pathogenesis of Type II diabetes mellitus. Diabetologia 2001;44:929-945

32. Lyssenko V, Almgren P, Anevski D, Perfekt R, Lahti K, Nissén M, Isomaa B, Forsen B, Homström N, Saloranta C, Taskinen MR, Groop L, Tuomi T; Botnia study group. Predictors of and longitudinal changes in insulin sensitivity and secretion preceding onset of type 2 diabetes. Diabetes 2005;54:166-174

33. Cnop M, Vidal J, Hull RL, Utzschneider KM, Carr DB, Schraw T, Scherer PE, Boyko EJ, Fujimoto WY, Kahn SE. Progressive loss of beta-cell function leads to worsening glucose tolerance in first-degree relatives of subjects with type 2 diabetes. Diabetes Care 2007;30:677-682

34. Stefan N, Haring HU. The metabolically benign and malignant fatty liver. Diabetes 2011;60:2011-2017

35. Kitabchi AE, Temprosa M, Knowler WC, Kahn SE, Fowler SE, Haffner SM, Andres R, Saudek C, Edelstein SL, Arakaki R, Murphy MB, Shamoon H; Diabetes Prevention Program Research Group. Role of insulin secretion and sensitivity in the evolution of type 2 diabetes in the diabetes prevention program: effects of lifestyle intervention and metformin. Diabetes 2005;54:2404-2414

36. de Mello VD, Lindström J, Eriksson J, Ilanne-Parikka P, Keinänen-Kiukaanniemi S, Sundvall J, Laakso M, Tuomilehto J, Uusitupa M. Insulin secretion and its determinants in the progression of impaired glucose tolerance to type 2 diabetes in impaired glucose-tolerant individuals: the Finnish Diabetes Prevention Study. Diabetes Care 2012;35:211-217

37. Schwarz PE, Greaves CJ, Lindström J, Yates T, Davies MJ. Nonpharmacological interventions for the prevention of type 2 diabetes mellitus. Nat Rev Endocrinol 2012;8:363-373

38. Johnson M, Jones R, Freeman C, Woods HB, Gillett M, Goyder E, Payne N. Can diabetes prevention programmes be translated effectively into real-world settings and still deliver improved outcomes? A synthesis of evidence. Diabet Med 2013;30:3-15

39. Papandonatos GD, Pan Q, Pajewski NM, Delahanty LM, Peter I, Erar B, Ahmad S, Harden M, Chen L, Fontanillas P; GIANT Consortium, Wagenknecht LE, Kahn SE, Wing RR, Jablonski KA, Huggins GS, Knowler WC, Florez JC, McCaffery JM, Franks PW; Diabetes Prevention Program and the Look AHEAD Research Groups. Genetic Predisposition to Weight Loss and Regain With Lifestyle Intervention: Analyses From the Diabetes Prevention Program and the Look AHEAD Randomized Controlled Trials. Diabetes 2015;64:4312-4321

40. Baum A, Scarpa J, Bruzelius E, Tamler R, Basu S, Faghmous J. Targeting weight loss interventions to reduce cardiovascular complications of type 2 diabetes: a machine learning-based post-hoc analysis of heterogeneous treatment effects in the Look AHEAD trial. Lancet Diabetes Endocrinol 2017;5:808-815

41. Barry E, Roberts S, Oke J, Vijayaraghavan S, Normansell R, Greenhalgh T. Efficacy and effectiveness of screen and treat policies in prevention of type 2 diabetes: systematic review and meta-analysis of screening tests and interventions. BMJ 2017;356:i6538

42. Diehl AM, Day C. Cause, Pathogenesis, and Treatment of Nonalcoholic Steatohepatitis. N Engl J Med 2017;377:2063-2072

43. Wagner R, Tabák ÁG, Fehlert E, Fritsche L, Jaghutriz BA, Bánhegyi RJ, Schmid SM, Staiger H, Machicao F, Peter A, Häring HU, Fritsche A, Heni M. Excessive fuel availability amplifies the FTO-mediated obesity risk: results from the TUEF and Whitehall II studies. Sci Rep 2017;7:15486

Table 1 Comparison of baseline parameters (mean±SD) of the low-risk versus high risk group (individuals with complete follow-up).

	LOW RISK (LR)	**HIGH RISK (HR)**	
sex female/male (%)	124/77 (62/38)	395/312 (56/44)	0.16
age (years)	57±11	59±10	0.057
weight (kg)	80.7±16.2	92.2±19.4	<0.0001
body mass index (kg m^{-2})	28.1±5.2	31.7±5.8	<0.0001
waist circumference (cm)	94±12	105±14	<0.0001
waist-to-hip ratio	0.89±0.08	0.94±0.08	<0.0001
systolic blood pressure (mmHg)	135±17	140±17	0.0013
diastolic blood pressure (mmHg)	84±11	86±11	0.043
fasting glucose (mmol l^{-1})	5.7±0.4	6.0±0.5	<0.0001
post-challenge glucose (mmol l^{-1})	6.8±1.5	7.8±1.7	<0.0001
glucose AUC (mmol min l^{-1})	934±121	1131±160	<0.0001
glycated hemoglobin (%)	5.6±0.3	5.8±0.3	<0.0001
glycated hemoglobin (mmol mol^{-1})	38.1±3.6	39.7±3.8	<0.0001
triglycerides (mmol l^{-1})	1.25±0.85	1.63±0.96	<0.0001
cholesterol (mmol l^{-1})	5.28±0.87	5.44±1.05	0.026
LDL cholesterol (mmol l^{-1})	3.17±0.81	3.34±0.91	0.013
HDL cholesterol (mmol l^{-1})	1.51±0.57	1.38±0.39	0.0025
Liver fat content (%)	2.85±2.92	10.45±8.19	<0.0001
Insulin sensitivity index (AU)	9.96±5.09	5.61±3.06	<0.0001
Insulin secretion (Disposition index)(AU)	1533±1187	671±467	<0.0001
hypertension no/yes (%)	119/72 (62/38)	307/366 (46/54)	<0.0001
Hyperlipidemia no/yes (%)	113/72 (61/39)	355/290 (55/45)	0.17
History of myocardial infarction no/yes (%)	188/4 (98/2)	639/17 (97/3)	0.89
History of stroke no/yes (%)	185/6 (97/3)	636/17 (97/3)	0.88
peripheral artery disease no/yes (%)	173/13 (93/7)	572/80 (88/12)	0.059
medication: angiotensine convertase inhibitors no/yes (%)	180/21 (90/10)	593/114 (84/16)	0.06
medication: angiotensine receptor blockers no/yes (%)	168/33 (84/16)	536/171 (76/24)	0.026
medication: thiazide diuretics no/yes (%)	185/16 (92/8)	607/100 (86/14)	0.028
medication: other diuretics no/yes (%)	196/5 (98/2)	676/31 (96/4)	0.31
medication: beta blockers no/yes (%)	174/27 (87/13)	545/162 (77/23)	0.0048
medication: statins no/yes (%)	175/26 (87/13)	581/126 (82/18)	0.13
current smoking no/yes (%)	184/10 (95/5)	645/44 (94/6)	0.64
alcohol consumption n (%)			0.054
1_none	31 (16)	71 (10)	
2_rarely	73 (37)	304 (45)	
3_week-ends	14 (7)	57 (8)	

4_weekely_2-3	60 (31)	169 (25)	
5_daily	18 (9)	84 (12)	
highest education n (%)			0.067
1_none	5 (3)	19 (3)	
2_post_secondary	99 (50)	314 (46)	
3_bachelor_or_equivalent	33 (17)	174 (26)	
4_master_or_equivalent	60 (30)	172 (25)	

Table 2: Changes of key study variables between baseline and follow up in low-risk (control vs conventional LI) and high-risk individuals (conventional vs intensive LI)

	Low Risk			High Risk		
	Control*	**Conventional***	**p-value****	**Conventional***	**Intensive***	**p-value****
number	101	100		351	356	
weight (kg)	-0.5 [-1.0;0.3]	-2.2 [-3.0;-1.4]	<0.0001	-2.5 [-3.2;-1.9]	-4.0 [-4.6;-3.3]	<0.0001
body mass index (kg m^{-2})	-0.2 [-0.5;0.1]	-0.8 [-1.1;-0.5]	<0.0001	-0.9 [-1.1;-0.7]	-1.3 [-1.6;-1.1]	<0.0001
fasting glucose (mmol l^{-1})	-0.07 [-0.17;-0.03]	-0.21 [-0.32;-0.11]	0.02	-0.17 [-0.23;-0.11]	-0.26 [-0.32;-0.19]	0.03
post-challenge glucose (mmol l^{-1})	-0.36 [-0.71;-0.00]	-0.54 [-0.89;-0.19]	0.4	-0.48 [-0.69;-0.28]	-0.77 [-0.98;-0.57]	0.03
glucose AUC (mmol min l^{-1})	-1 [-33;31]	-31 [-62;1]	0.1	-66 [-85;-46]	-92 [-111;-73]	0.03
glycated hemoglobin (%)	-0.0 [-0.1;0.0]	-0.0 [-0.1;0.0]	0.6	-0.1 [-0.1;-0.1]	-0.1 [-0.1;-0.2]	0.02
glycated hemoglobin (mmol/mol)	-0.3 [-1.0;0.3]	-0.5 [-0.9;0.2]	0.6	-1.0 [-1.5;-0.7]	-1.5 [-0.8;-1.1]	0.02
Insulin sensitivity index (AU)	-0.7 [-1.6;0.2]	0.3 [-0.6;1.1]	0.06	1.3 [0.9;1.7]	2.0 [1.6;2.4]	0.01
Insulin secretion (Disposition ind) (AU)	-198 [-459;63]	-46 [-307;216]	0.3	247 [151;343]	260 [166;355]	0.8
Liver fat content (%) ***	0.0 [-0.5;0.6]	-0.2 [-0.8;0.4]	0.4	-2.6 [-3.3;-1.8]	-3.9 [-4.6;-3.2]	0.002
Framingham 10-year-CV-risk (%)	-0.4 [-1.9;1.0]	-1.0 [-2.4;0.4]	0.5	-2.0 [-3.0;-0.9]	-3.8 [-4.9;-2.8]	0.007

* Least-Square Means [95% CI)] of changes from baseline to follow up (1 year) ** ANCOVA adjusted for baseline and center *** measured at both baseline and follow up in n=631 individuals. LR-CTRL: n=72, LR-CONV: n=74, HR-CONV: n=241, HR-INT: n=244

Figures Legends

Figure 1
Participant flow during the study (Consort diagram).

Figure 2
Plasma glucose levels at 120 minutes after standardized 75 g glucose challenge (panel A), and insulin sensitivity (panel B) at baseline, 6 and 12 months during LI, hepatic fat content (panel C), and cardiometabolic risk (panel D) at baseline and 12 months during LI. Values shown as least-square means (LSM) with standard errors, adjusted for study center.
# indicates significant difference ($p<0.05$) between HR-CONV and HR-INT in change of the parameter from baseline.

Figure 3
Results after 3 years observation (1 year of lifestyle intervention) and additional 2 years of follow up). Cumulative frequency of normal glucose tolerance in individuals with low risk (left panel, log-rank test $p=0.03$) and high risk (right panel, log-rank test 0.008).
The inserts represent parametric survival models using fits of interval censored data. $p=0.01$ for the conventional vs. control intervention in the low-risk group (left panel) and $p=0.003$ for the intensive vs conventional intervention in the high risk group (right panel).

Figure 1

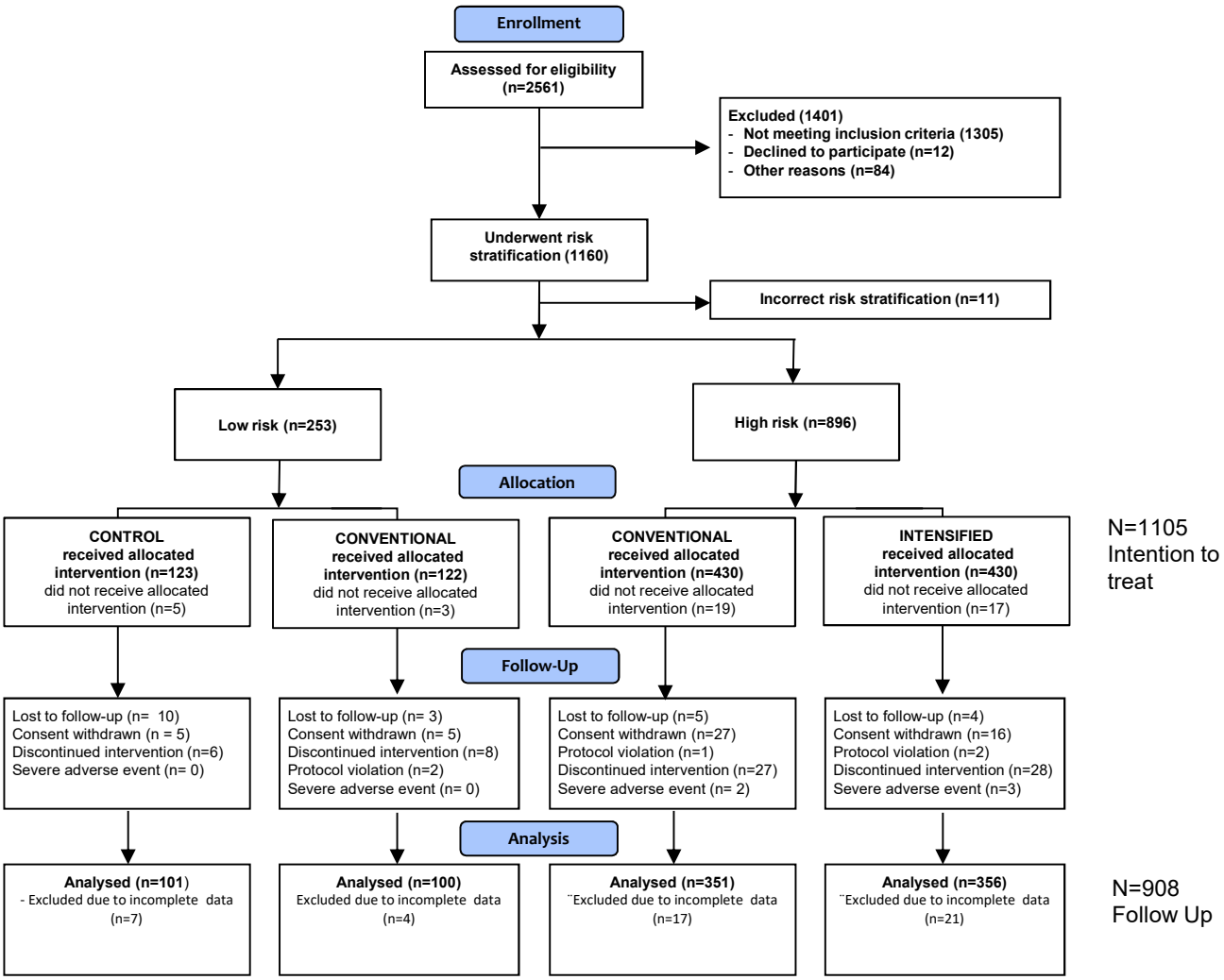
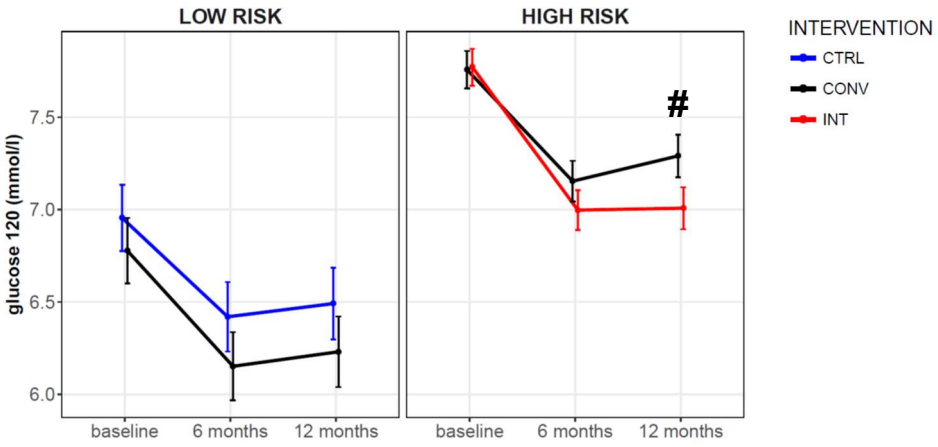
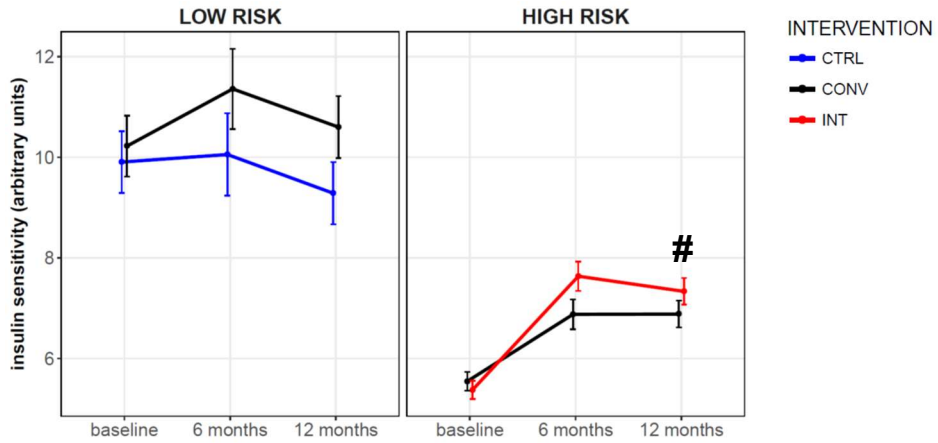

Enrollment
Assessed for eligibility (n=2561)
Excluded (1401)
- Not meeting inclusion criteria (1305)
- Declined to participate (n=12)
- Other reasons (n=84)
Underwent risk stratification (1160)
Incorrect risk stratification (n=11)
Low risk (n=253)
High risk (n=896)
Allocation
CONTROL received allocated intervention (n=123) did not receive allocated intervention (n=5)
CONVENTIONAL received allocated intervention (n=122) did not receive allocated intervention (n=3)
CONVENTIONAL received allocated intervention (n=430) did not receive allocated intervention (n=19)
INTENSIFIED received allocated intervention (n=430) did not receive allocated intervention (n=17)
N=1105 Intention to treat
Follow-Up
Lost to follow-up (n= 10) Consent withdrawn (n = 5) Discontinued intervention (n=6) Severe adverse event (n= 0)
Lost to follow-up (n= 3) Consent withdrawn (n= 5) Discontinued intervention (n=8) Protocol violation (n=2) Severe adverse event (n= 0)
Lost to follow-up (n=5) Consent withdrawn (n=27) Protocol violation (n=1) Discontinued intervention (n=27) Severe adverse event (n= 2)
Lost to follow-up (n=4) Consent withdrawn (n=16) Protocol violation (n=2) Discontinued intervention (n=28) Severe adverse event (n=3)
Analysis
Analysed (n=101) - Excluded due to incomplete data (n=7)
Analysed (n=100) Excluded due to incomplete data (n=4)
Analysed (n=351) ¨Excluded due to incomplete data (n=17)
Analysed (n=356) ¨Excluded due to incomplete data (n=21)
N=908 Follow Up

Figure 2

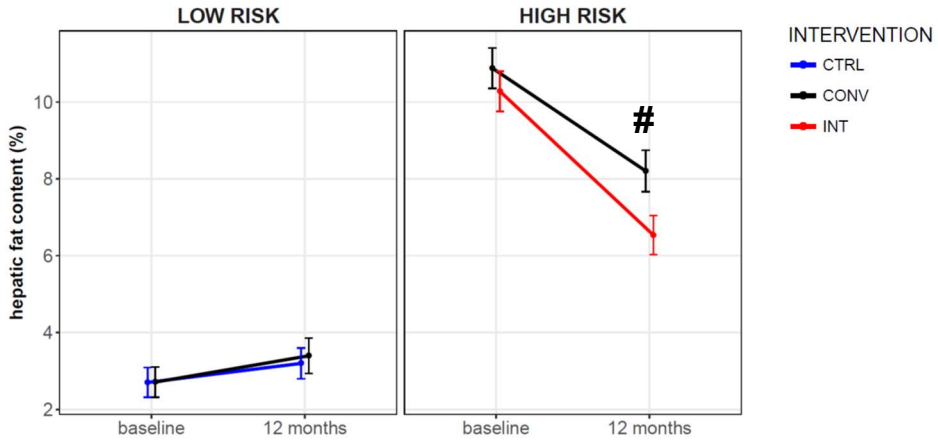
A



B



C



D

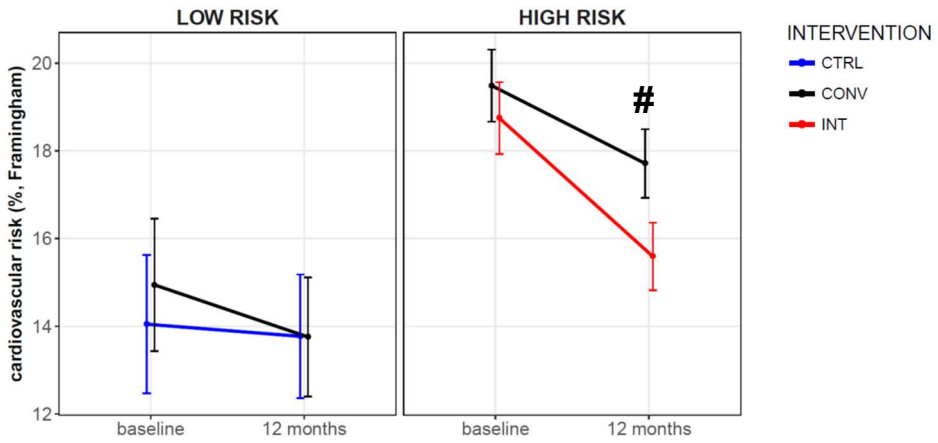
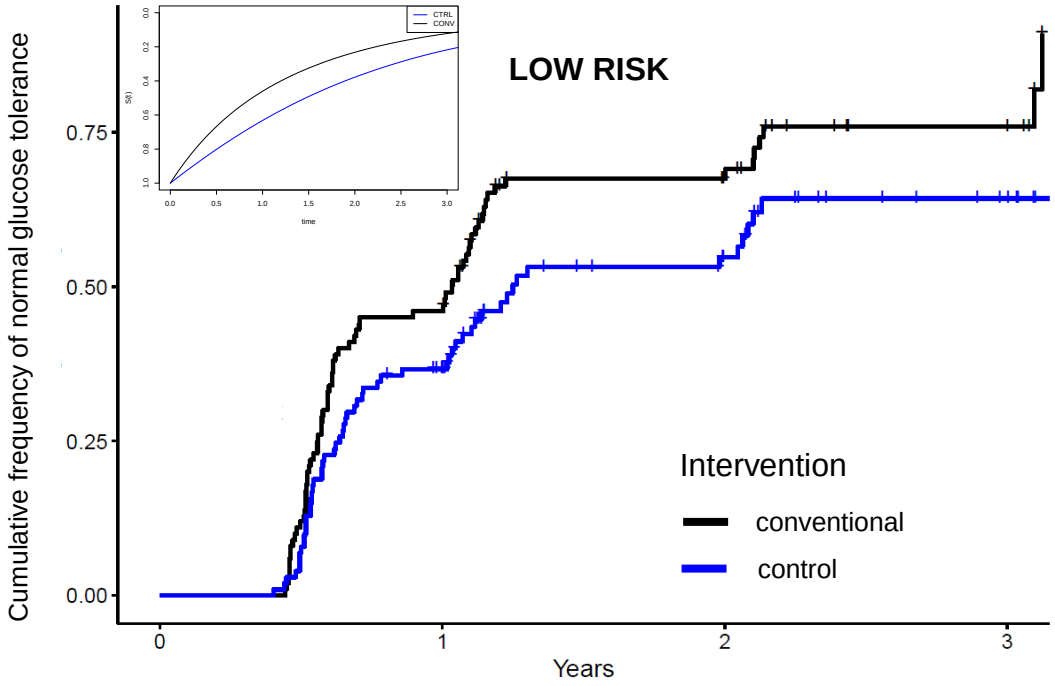
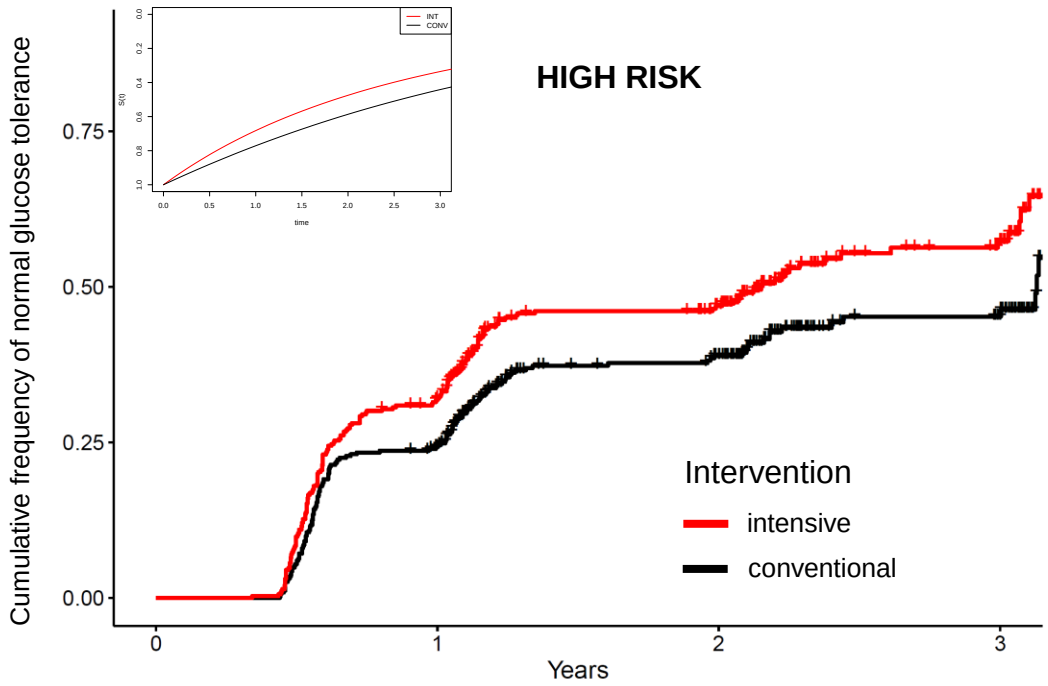


Figure 3





Numbers at chance to convert to NGT

conventional	100	54	21	7
control	101	60	26	6

Numbers at chance to convert to NGT

intensive	356	238	136	40
conventional	351	260	149	51

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

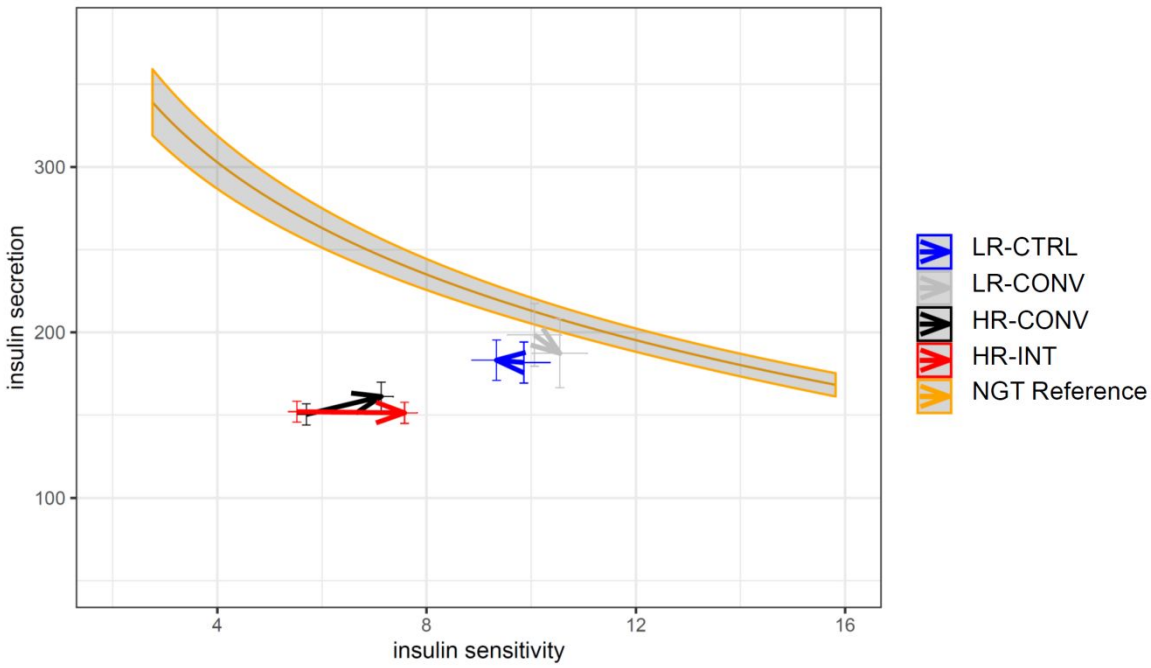
Supplement to:

Fritsche A, Wagner R, Heni M et al.

Different effects of lifestyle intervention in high- and low-risk prediabetes -
Results of the randomized controlled Prediabetes Lifestyle Intervention Study (PLIS)

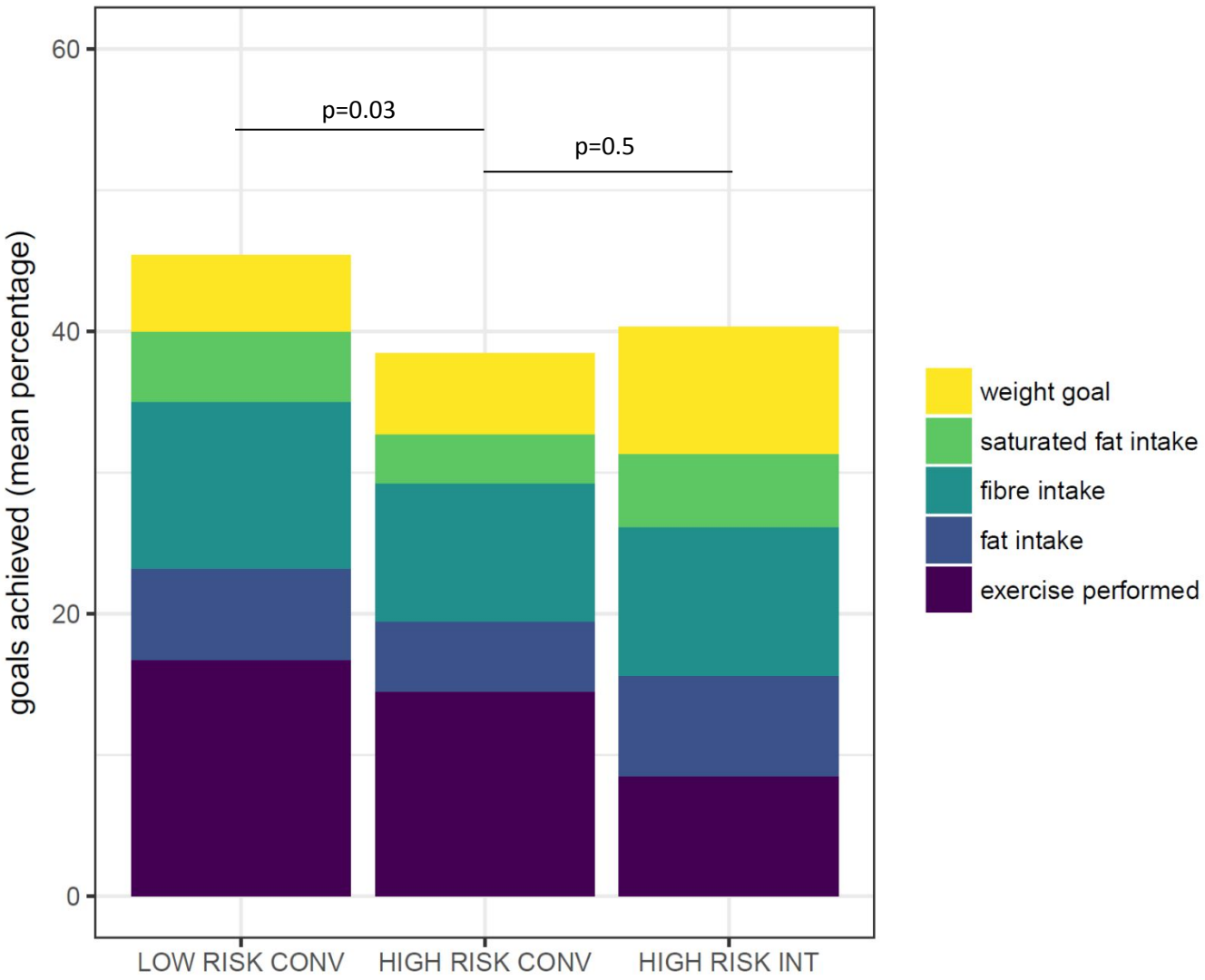
Table of Contents

Appendix Figure 1



Change in insulin secretion and sensitivity during the study in the different study arms. Insulinogenic index (arbitrary units) as marker for insulin secretion is shown as hyperbolic function of insulin sensitivity (insulin sensitivity index, arbitrary units, unadjusted values). Subjects with normal glucose tolerance (NGT, age 18-50 years) from the German TUEF study (43) were used to compute the hyperbolic function (n=1421). The arrows represent baseline values (origin) and values after 12 months of intervention (end) in the study arms.
95 % CI are shown, blue: LR-CTRL, grey: LR-CONV, black: HR-CONV, red: HR-INT.

Appendix Figure 2



Aggregate percentage of completed lifestyle goals during the study.

To scale the aggregate percentage of all lifestyle goals to a maximum of 100%, the number of completed individual goals are downscaled by a factor of 5 (number of individual goals). The aggregate percentage of all completed lifestyle goals was higher in the LR-CONV compared to the HR-CONV (p=0.03, Wilcoxon-test) and not different between HR-CONV and HR-INT (p=0.5).

Appendix Table 1
Participation at different academic diabetes centers where the study was performed.

From each study center, about 2 fold of actually included individuals with prediabetes were potentially eligible for the study. For all study centers, a total of n=2561 were potentially eligible.

	Number of participants receiving allocated intervention	Number of participants completing follow up
University Hospital of Tübingen	351	304
Universiy Hospital of Dresden	213	178
German Institute of Human Nutrition, Potsdam	175	137
University Hospital of Heidelberg	104	80
University of Düsseldorf	59	48
Technical University of Munich	106	82
Ludwig Maximilian University Munich	49	40
University Hospital of Leipzig	48	39

Appendix Table 2
Exclusion criteria.

Criterion	Specific definition
Pregnancy	
Lactation	
Symptomatic coronary artery disease	
Active malignant disease	Active malignant disease and/or unintended weight loss > 10% over the last 6 months
Elevated liver transaminases	3 times above the upper limit of normal level
Chronic kidney disease	estimated glomerular filtration ratio < 50 ml/min/1.73m^2
Systemic infection	
Glucocorticoid use	
Severe mental illness	

Appendix Table 3

Full-set analysis on all 1105 participants

using multivariable imputation performed on a wide-dataset encompassing basic variables (sex, age, BMI, waist circumference, education, study center), and glycemic variables (glucose during OGTT, AUC glucose, HbA1c), variables on insulin secretion and sensitivity (ISI and IGI), disposition index as well as liver fat content at the visits at 6 months and 12 months. Missing data were imputed for all visits. The imputation was performed using the MICE package in R using default settings (predictive mean matching as default algorithm, 5 iterations) and passive imputation for derived variables (disposition indexes).

		LOW RISK			HIGH RISK		
	Imputed variables (baseline/ follow up)	**LR-CTRL**	**LR-CONV**	**p-value**	**HR-CONV**	**HR-INT**	**p-value**
body mass index (kg m^{-2})	0/157	0 [-0.2;0.2]	-0.6 [-0.9;-0.4]	<0.0001	-1.1 [-1.3;-1]	-1.6 [-1.8;-1.4]	<0.0001
fasting glucose (mmol l^{-1})	1/159	-0.13 [-0.21;-0.05]	-0.29 [-0.37;-0.21]	0.004	-0.2 [-0.25;-0.15]	-0.29 [-0.34;-0.24]	0.02
post-challenge glucose (mmol l^{-1})	2/164	-0.49 [-0.76;-0.21]	-0.45 [-0.73;-0.18]	0.9	-0.65 [-0.82;-0.48]	-0.94 [-1.11;-0.77]	0.02
glucose AUC (mmol min l^{-1})	1/163	-8 [-32;17]	-14 [-38;11]	0.7	-76 [-92;-61]	-105 [-120;-89]	0.01
glycated hemoglobin (%)	1/161	0 [0;0]	0 [-0.1;0]	0.2	-0.1 [-0.1;-0.1]	-0.1 [-0.2;-0.1]	0.02
Insulin sensitivity index (AU)	25/181	-1.2 [-1.8;-0.6]	-0.2 [-0.9;0.4]	0.04	1.2 [0.9;1.5]	1.9 [1.6;2.2]	0.004
Insulin secretion (Disposition ind) (AU)	26/184	-236 [-434;-37]	-247 [-446;-48]	0.9	127 [51;203]	168 [92;244]	0.5
Liver fat content (%)	184/437	0.1 [-0.3;0.5]	-0.1 [-0.5;0.3]	0.4	-3.1 [-3.5;-2.6]	-4.5 [-4.9;-4]	<0.0001

Appendix Table 4:
Comparison of baseline parameters (mean±SD), low-risk group control versus conventional intervention and high risk group conventional versus intensive intervention.

	LR-CTRL	LR-CONV	HR-CONV	HR-INT
sex female/male (%)	63/38 (62/38)	61/39 (61/39)	186/165 (53/47)	209/147 59/41)
age (years)	57±12	58±11	59±10	59±10
weight (kg)	80.1±16.1	81.3±16.3	92±19.7	92.4±19.2
body mass index (kg m^{-2})	27.9±5.1	28.3±5.3	31.5±5.9	31.9±5.7
waist circumference (cm)	93±12	94±13	105±14	105±14
waist-to-hip ratio	0.89±0.09	0.89±0.08	0.94±0.09	0.94±0.08
systolic blood pressure (mmHg)	135±16	135±17	140±16	139±17
diastolic blood pressure (mmHg)	84±10	85±12	86±10	86±11
fasting glucose (mmol l^{-1})	5.7±0.5	5.7±0.4	5.9±0.5	6.0±0.5
post-challenge glucose (mmol l^{-1})	6.9±1.4	6.7±1.5	7.8±1.7	7.8±1.7
glucose AUC (mmol min l^{-1})	935±112	933±130	1131±160	1131±161
glycated hemoglobin (%)	5.7±0.3	5.6±0.3	5.8±0.3	5.8±0.4
glycated hemoglobin (mmol mol^{-1})	38.4±3.6	37.8±3.6	40.1±3.5	39.4±4.1
triglycerides (mmol l^{-1})	1.26±0.96	1.24±0.72	1.64±0.95	1.63±0.98
cholesterol (mmol l^{-1})	5.34±0.86	5.22±0.87	5.46±1.09	5.43±1.02
LDL cholesterol (mmol l^{-1})	3.23±0.83	3.12±0.79	3.38±0.95	3.3±0.87
HDL cholesterol (mmol l^{-1})	1.47±0.4	1.56±0.71	1.37±0.36	1.39±0.41
Liver fat content (%)	2.85±2.74	2.86±3.1	10.72±8.76	10.18±7.57
Insulin sensitivity index (AU)	9.86±5.05	10.06±5.16	5.70±3.12	5.52±3.01
Insulin secretion (Disposition index) (AU)	1440±994	1627±1352	654±424	688±506
Hypertension no/yes (%)	56/39 (59/41)	63/33 (66/34)	155/185 (46/54)	152/181 (46/54)

Hyperlipidemia no/yes (%)	55/38 (59/41)	58/34 (63/37)	184/145 (56/44)	171/145 (54/46)
History of myocardial infarction no/yes (%)	91/3 (97/3)	97/1 (99/1)	325/7 (98/2)	314/10 (97/3)
History of stroke no/yes (%)	90/4 (96/4)	95/2 (98/2)	325/4 (99/1)	311/13 (96/4)
peripheral artery disease no/yes (%)	85/6 (93/7)	88/7 (93/7)	288/41 (88/12)	284/39 (88/12)
medication: angiotensine convertase inhibitors no/yes (%)	89/12 (88/12)	91/9 (91/9)	290/61 (83/17)	303/53 (85/15)
medication: angiotensine receptor blockers no/yes (%)	84/17 (83/17)	84/16 (84/16)	276/75 (79/21)	260/96 (73/27)
medication: thiazide diuretics no/yes (%)	93/8 (92/8)	92/8 (92/8)	306/45 (87/13)	301/55 (85/15)
medication: other diuretics no/yes (%)	98/3 (97/3)	98/2 (98/2)	335/16 (95/7)	341/15 (96/4)
medication: beta blockers no/yes (%)	88/13 (87/13)	86/14 (86/14)	272/79 (77/23)	273/83 (77/23)
medication: statins no/yes (%)	89/12 (88/12)	86/14 (86/14)	287/64 (82/18)	294/62 (83/17)
current smoking no/yes (%)	93/3 (97/3)	91/7 (93/7)	324/22 (94/6)	321/22 (94/6)
alcohol consumption n (%)				
1_none	20 (21)	11 (11)	29 (8)	42 (12)
2_rarely	30 (31)	43 (44)	157 (45)	147 (43)
3_week-ends	9 (9)	5 (5)	33 (10)	24 (7)
4_weekely_2-3	33 (34)	27 (27)	82 (24)	87 (26)
5_daily	5 (5)	13 (13)	44 (13)	40 (12)
highest education n (%)				
1_none	1 (1)	4 (4)	8 (2)	11 (3)
2_post_secondary	46 (47)	53 (54)	160 (48)	154 (45)
3_bachelor_or_equivalent	18 (18)	15 (15)	85 (25)	89 (26)
4_master_or_equivalent	34 (34)	26 (27)	84 (25)	88 (26)

Appendix Table 5

Comparison of baseline variables (mean±SD) non-completers versus completers.

	Non-completer (n=197)	Completer (n=908)	p-value
Riskgroup n (%)			1
1_low risk	44 (22)	201 (22)	
2_high risk	153 (78)	707 (78)	
Intervention group n (%)			0.91
control	22 (11)	101 (11)	
conventional	101 (51)	451 (50)	
intensive	74 (38)	356 (39)	
sex female/male (%)	132/65 (67/33)	519/389 (57/43)	0.014
age (years)	54±12	59±10	<0.0001
weight (kg)	93.1±21.1	89.6±19.4	0.036
body mass index (kg m^{-2})	32.2±6.5	30.9±5.9	0.0091
waist circumference (cm)	104±16	102±14	0.19
waist-to-hip ratio	0.92±0.09	0.93±0.09	0.19
systolic blood pressure (mmHg)	136±16	139±17	0.048
diastolic blood pressure (mmHg)	86±10	86±11	0.98
fasting glucose (mmol l^{-1})	6.0±0.6	5.9±0.5	0.089
post-challenge glucose (mmol l^{-1})	7.5±1.9	7.6±1.7	0.7
glucose AUC (mmol min l^{-1})	1084±191	1088±173	0.79
glycated hemoglobin (mmol mol^{-1})	39.3±4.4	39.4±3.8	0.72
glycated hemoglobin (%)	5.7±0.4	5.8±0.3	0.72
triglycerides (mmol l^{-1})	1.53±0.86	1.55±0.95	0.83
cholesterol (mmol l^{-1})	5.33±0.98	5.41±1.01	0.31
LDL cholesterol (mmol l^{-1})	3.26±0.88	3.3±0.89	0.52
HDL cholesterol (mmol l^{-1})	1.39±0.38	1.41±0.44	0.59
Hepatic triglyceride content (%)	9.12±8.21	8.75±8	0.62
Insulin sensitivity index	6.38±3.68	6.58±4.04	0.52
Insulin secretion (Disposition index) (AU)	882.±660	863±781	0.73
Hypertension no/yes (%)	95/84 (53/47)	426/438 (49/51)	0.4
hyperlipidemia no/yes (%)	108/64 (63/37)	468/362 (56/44)	0.14
myocardial infarction no/yes (%)	172/4 (98/2)	827/21 (98/2)	1
stroke no/yes (%)	176/2 (99/1)	821/23 (97/3)	0.32
peripheral artery disease no/yes (%)	154/21 (88/12)	745/93 (89/11)	0.83
medication: angiotensine convertase inhibitors no/yes (%)	171/26 (87/13)	773/135 (85/15)	0.62
medication: angiotensine receptor blockers no/yes (%)	160/37(81/19)	704/204 (78/22)	0.3
medication: thiazide diuretics no/yes (%)	176/21 (89/11)	792/116 (87/13)	0.49
medication: other diuretics no/yes (%)	194/3 (98/2)	872/36 (96/4)	0.14
medication: beta blockers no/yes (%)	156/41 (79/21)	719/189 (79/21)	1

medication: statins no/yes (%)	173/24 888/12)	756/152 (83/17)	0.14
current smoking no/yes (%)	154/26 (86/14)	829/54 (94/6)	0.00021
alcohol use			0.025
1_none	31 (17)	102 (12)	
2_rarely	89 (50)	377 (42)	
3_week-ends	13 (7)	71 (8)	
4_weekely_2-3	31 (17)	229 (26)	
5_daily	16 (9)	102 (12)	
highest education			0.62
1_none	6 (3)	24 (3)	
2_post_secondary	95 (52)	413 (47)	
3_bachelor_or_equivalent	38 (21)	207 (24)	
4_master_or_equivalent	44 (24)	232 (26)	

Appendix Table 6

Effect of conventional LI in low risk individuals versus high risk individuals.

*ANCOVA, adjusted for baseline and center.

	Between-group difference of conventional intervention * (for HR-CONV, reference: LR-CONV beta coefficient (±SE))	**p-value**
age (years)	-0 (±0)	0.6
weight (kg)	0.4 (±0.6)	0.5
body mass index (kg m^{-2})	0.1 (±0.2)	0.5
waist circumference (cm)	-1 (±1)	0.6
waist-to-hip ratio	-0.02 (±0.01)	0.008
lean mass percent	-5.5 (±2.3)	0.02
fat mass percent	0.9 (±0.5)	0.07
habitual physical activity score	0.1 (±0.1)	0.5
systolic blood pressure (mmHg)	-3 (±2)	0.09
diastolic blood pressure (mmHg)	-1 (±1)	0.3
heart rate (1/min)	-0 (±1)	0.7
fasting glucose (mmol l^{-1})	-0.16 (±0.06)	0.006
post-challenge glucose (mmol l^{-1})	-0.55 (±0.20)	0.008
glucose AUC (mmol min l^{-1})	-33 (±20)	0.1
glycated hemoglobin (mmol mol^{-1})	-0.0 (±0.4)	1
glycated hemoglobin (%)	-0.0 (±0.0)	1
triglycerides (mmol l^{-1})	0.11 (±0.07)	0.1
cholesterol (mmol l^{-1})	0.12 (±0.08)	0.1
LDL cholesterol (mmol l^{-1})	0.09 (±0.07)	0.2
HDL cholesterol (mmol l^{-1})	0.04 (±0.03)	0.2
C-reactive protein (mg dl^{-1})	0.1 (±0.8)	0.9
Aspartate aminotransferase (Units l^{-1})	0.1 (±1.4)	0.9
Alanin aminotransferase (Units l^{-1})	0.3 (±1.5)	0.8
Gamma glutamyltransferase (Units l^{-1})	2.4 (±2.1)	0.3
Insulin sensitivity index	-0.1 (±0.5)	0.9
Insulin secretion (Disposition index) (AU)	226 (±134)	0.09
Liver fat content (%)	0.1 (±0.6)	0.9
Framingham 10-year-CV-risk (%)	-0.4 (±1.0)	0.7

Calculations

$$Insulin\ sensitivity\ index\ (Matsuda - ISI) = \frac{10000}{\sqrt{(G_0 * I_0) * (G_{mean} * I_{mean})}}$$

$$Insulinogenic\ Index: IGI = \frac{\Delta I_{0,30}}{\Delta G_{0,30}}$$

$$Disposition\ Index: (Insulin\ sensitivity\ index) * (Insulinogenic\ index)$$

G_{mean} = *mean plasma glucose 0,30,60,90,120 min during OGTT*

I_{mean} =*mean plssma glucose 0,30,60,90,120 min during OGTT*

G_0 = *fasting plasma glucose,* I_0 = *fasting plasma insulin*

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Data and Resource Availability

Due to ethical regulations, we cannot share individual participant data. Mean values and confidence intervals of all analysed patient-level data are available and maybe shared upon reasonable request. Study protocol, statistical analysis plan and analytic code used to generate results are available upon reasonable request.

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