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

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Received: 11 April 2017 / Accepted: 11 July 2017 / Published online: 24 August 2017
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Abstract

Aims/hypothesis Lifestyle intervention is effective to prevent type 2 diabetes. However, a considerable long-term non-response occurs to a standard lifestyle intervention. We investigated which risk phenotypes at baseline and their changes during the lifestyle intervention predict long-term glycaemic non-response to the intervention.

Methods Of 300 participants at high risk for type 2 diabetes who participated in a 24 month lifestyle intervention with diet modification and increased physical activity, 190 participants could be re-examined after 8.7 ± 1.6 years. All individuals underwent a five-point 75 g OGTT and measurements of body fat compartments and liver fat content with MRI and spectroscopy at baseline, 9 and 24 months during the lifestyle intervention, and at long-term follow-up. Fasting proinsulin to insulin conversion (PI/I ratio) and insulin sensitivity and secretion were calculated from the OGTT. Non-response to lifestyle

intervention was defined as no decrease in glycaemia, i.e. no decrease in AUC for glucose at 0–120 min during OGTT ($AUC_{\text{glucose}_{0-120 \text{ min}}}$).

Results Before the lifestyle intervention, 56% of participants had normal glucose regulation and 44% individuals had impaired fasting glucose and/or impaired glucose tolerance. At long-term follow-up, 11% had developed diabetes. Multivariable regression analysis with adjustment for age, sex, BMI and change in BMI during the lifestyle intervention revealed that baseline insulin secretion and insulin sensitivity, as well as change in insulin sensitivity during the lifestyle intervention, predicted long-term glycaemic control after 9 years. In addition, increased hepatic lipid content as well as impaired fasting proinsulin conversion at baseline were newly detected phenotypes that independently predicted long-term glycaemic control.

Conclusions/interpretation Increased hepatic lipid content and impaired proinsulin conversion are new predictors, independent of change in body weight, for non-response to lifestyle intervention in addition to the confirmed factors, impaired insulin secretion and insulin sensitivity.

Electronic supplementary material The online version of this article (doi:10.1007/s00125-017-4407-z) contains peer-reviewed but unedited supplementary material, which is available to authorised users.

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Keywords Fatty liver · Insulin secretion · Insulin sensitivity · Lifestyle intervention · Prediabetes · Predictors · Proinsulin

Abbreviations

$AUC_{\text{glucose}_{0-120 \text{ min}}}$	AUC for glucose at 0–120 min during OGTT
DPP	Diabetes Prevention Program
DPPOS	Diabetes Prevention Program Outcome Study
DPS	Diabetes Prevention Study

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¹ H-MRS	Proton magnetic resonance spectroscopy
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IGI	Insulinogenic index
ISI	Insulin sensitivity index
NAFLD	Non-alcoholic fatty liver disease
PI/I ratio	Proinsulin/insulin ratio
SCAT	Subcutaneous adipose tissue
TAT	total adipose tissue
TULIP	Tübingen Lifestyle Intervention Program
VAT	Visceral adipose tissue

Introduction

Lifestyle intervention has repeatedly and conclusively been shown to prevent or postpone the development of type 2 diabetes in high-risk individuals [1–9]. Long-term follow-up of several studies has also been reported [4, 6, 7]. The Finnish Diabetes Prevention Study (DPS) showed a relative diabetes risk reduction of up to 38% after 13 years of intervention, compared with a control group [7]. The Diabetes Prevention Program (DPP) study and the Da Qing Diabetes Prevention study also showed a significant reduction in diabetes risk (34–43%) compared with the control group, lasting for 10 to 20 years after the lifestyle intervention [4, 6].

However, the effectiveness of a standard lifestyle intervention on glucose metabolism is highly variable, and a substantial number of participants develop diabetes [1, 3, 10]. Impaired insulin secretion as well as insulin resistance are well-established pathophysiological mechanisms in the development of type 2 diabetes [11–14]. Recently, we found that, besides baseline impaired insulin secretion, the presence of an insulin-resistant fatty liver is an important predictor of the short-term response to a standard lifestyle intervention [15]. Sufficient data are still lacking to elucidate the contribution of fatty liver to the long-term effects of a lifestyle intervention [11].

It is well accepted that, besides baseline insulin sensitivity and insulin secretion, the changes in these variables during a lifestyle intervention may play a role in its success [16–19].

Perreault et al showed in the US Diabetes Prevention Program Outcome Study (DPPOS) that the regression from impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) to a normal glucose regulation, which was strongly associated with improvement of BMI and insulin sensitivity during the lifestyle intervention, was strongly associated with a reduced incidence of diabetes [20, 21].

It is well accepted that fasting proinsulin, proinsulin to insulin conversion (PI/I ratio) and proinsulin to C-peptide ratio are predictors of the incidence of type 2 diabetes [22–30]. It is of great interest that lifestyle intervention in individuals with

IFG and/or IGT seems to have only a slight influence on insulin secretion [31, 32]. One aspect of beta cell dysfunction in addition to insulin secretion is the conversion of proinsulin to insulin (PI/I ratio); where the conversion is adversely affected, the ratio increases and the individual develops hyperproinsulinaemia [33]. An increased fasting PI/I ratio, indicating impaired proinsulin conversion, has been observed in individuals with IGT and type 2 diabetes [34, 35]. Data on the impact of lifestyle intervention on fasting PI/I ratio are rare [36–38], but those available indicate that proinsulin conversion is improved after lifestyle intervention.

The Tübingen Lifestyle Intervention Program (TULIP; trial registration number KFO 114 [<http://gepris.dfg.de/gepris/projekt/5396893?language=en>]) was initiated in 2003 in a population at high risk for type 2 diabetes in Germany. All participants underwent a 24 month lifestyle intervention programme. One unique feature of the TULIP study is that participants repeatedly underwent whole-body MRI and ¹H magnetic resonance spectroscopy (¹H-MRS) before the lifestyle intervention and at the follow-up visits. This allows for the first time the precise assessment of liver fat content and body fat composition (total adipose tissue [TAT], visceral adipose tissue [VAT], subcutaneous adipose tissue [SCAT]) before, during and after the lifestyle intervention [10, 39–41].

We have performed a long-term follow-up of participants from the TULIP study, with the aim of identifying the risk phenotypes at baseline and changes during the lifestyle intervention that predict the long-term glycaemic non-response to the intervention. This information is important to identify early those individuals who will probably not benefit from lifestyle intervention and offer them alternative treatment.

Methods

Participants and study design Individuals at high risk for type 2 diabetes from the southern part of Germany participated in TULIP, a prospective uncontrolled intervention study. They were included in the study when they fulfilled at least one of the following criteria: a family history of type 2 diabetes; BMI > 27 kg/m²; IGT (blood glucose ≥ 7.78 mmol/l); or previous diagnosis of gestational diabetes. Exclusion criteria were the diagnosis of diabetes mellitus or the presence of a severe critical mental or physical illness. Informed written consent was obtained from all participants and the local medical ethics committee approved the protocol.

A total of 413 individuals were included. At baseline, medical history was obtained and a physical examination performed. Phenotypic characterisation was performed using a 75 g OGTT, whole-body MRI and ¹H-MRS, as well as a cycle exercise test (see below). These examinations were repeated at 9 and 24 months, and after the long-term follow-up period of about 9 years.

After the baseline examination, all participants underwent 15 sessions of standardised individual face-to-face dietary counselling by trained diabetes educators. In the first 9 months of intervention, i.e. the intensive lifestyle intervention phase of the study, monthly visits were scheduled. The moderate maintenance phase of the lifestyle intervention, comprising 3-monthly visits, lasted from the ninth month until the end of the second year.

Counselling aimed to reduce body weight by $\geq 5\%$. Furthermore, participants were recommended to reduce the energy intake from fat to $< 30\%$ of total energy consumed, and to increase dietary fibre intake to at least 15 g/4185 kJ (1000 kcal). They were also encouraged to reduce the intake of saturated fatty acids to $< 10\%$ of total fat. During each visit, participants presented a 3 day food diary and discussed it with the dietitians. Diet composition was estimated with a validated software, using two representative days of the 3 day diary (DGE-PC 3.0; Deutsche Gesellschaft für Ernährung, Bonn, Germany). Individuals were motivated to perform at least 3 h moderate exercise per week. A heart rate monitor (Polar, Büttelbronn, Germany) was given to the participants and they were instructed to exercise so that their heart rate was just below their individual anaerobic threshold. Individual anaerobic threshold was measured by a motorised treadmill (Saturn, HP-Cosmos, Traunstein, Germany).

OGTT All individuals underwent a frequently sampled 75 g OGTT at baseline, after 9 months, after 24 months and at the long-term follow-up appointment. For the determination of plasma glucose and insulin levels, venous blood samples were obtained at 0, 30, 60, 90 and 120 min. Plasma glucose was measured using a bedside glucose analyser (YSI, Yellow Springs, CO, USA). AUC for glucose at 0–120 min during OGTT ($\text{AUC}_{\text{glucose}0-120 \text{ min}}$) was calculated with the trapezoid method. Plasma insulin and C-peptide were determined by an immunoassay with the ADVIA Centaur XP Immunoassay System (Siemens Healthcare Diagnostics, Erlangen, Germany).

Serum proinsulin concentrations were measured using a microparticle enzyme immunoassay (IBL, Hamburg, Germany) on a BEP III System (Siemens Healthcare, Erlangen, Germany). The proinsulin assay measures intact proinsulin and has 0% cross-reactivity with human insulin and C-peptide. There is cross-reactivity with the relevant proinsulin split products: des-31,32-proinsulin, 0.9%; des-32,33-proinsulin, 1.5%; des-64,65-proinsulin, 53%; and des-65,66-proinsulin, 55% [42]. Insulin sensitivity was calculated from glucose and insulin values during the OGTT as proposed by Matsuda and DeFronzo (i.e. to calculate the insulin sensitivity index [ISI]) [43]. Insulin secretion was estimated using the insulinogenic index (IGI) across 30 min ($\text{IGI}_{0-30 \text{ min}}$), calculated as $\text{Ins}_{30} - \text{Ins}_0 / \text{Glc}_{30} - \text{Glc}_0$, where Ins_0 and Glc_0 is insulin and glucose at 0 min, and Ins_{30} and Glc_{30} is insulin and

glucose at 30 min [17]. The relative insulin secretion (disposition index), which describes the insulin secretion relative to insulin sensitivity, was calculated as $\text{ISI} \times \text{IGI}_{0-30 \text{ min}}$ [17]. Fasting proinsulin to insulin conversion was determined by dividing $\text{proinsulin}_{0 \text{ min}}$ by $\text{insulin}_{0 \text{ min}}$ (PI/I ratio), where $\text{proinsulin}_{0 \text{ min}}$ is proinsulin at 0 min and $\text{insulin}_{0 \text{ min}}$ is insulin at 0 min. Fasting proinsulin to C-peptide ratio was determined by dividing $\text{proinsulin}_{0 \text{ min}}$ by C-peptide at 0 min ($\text{C-peptide}_{0 \text{ min}}$) [28].

T1-weighted MRI to measure body fat Determination of whole-body fat distribution was performed using an axial T1-weighted fast spin echo technique as previously described using a 1.5 T whole-body imager (Magnetom Sonata, Siemens Medical Solutions, Erlangen, Germany) [14, 40].

^1H -MRS to measure intrahepatic fat content Liver fat content was quantified by localised ^1H -MRS as previously described [14, 40]. Non-alcoholic fatty liver disease (NAFLD) was defined as liver fat content $\geq 5.56\%$ [44].

Maximal oxygen consumption The individuals underwent a continuous, incremental exercise test on a cycle ergometer to volitional exhaustion. The cycle ergometer test was performed on an electromagnetically braked cycle ergometer (Ergometrics 800 S; Ergoline, Bitz, Germany). Oxygen consumption was measured using spirometry (Cortex MetaLyzer, Metasoft 3.9, Leipzig, Germany). Maximal oxygen consumption ($\dot{V}\text{O}_{2\text{max}}$) is expressed as $\dot{V}\text{O}_{2\text{max}}$ per kg lean body mass ($\text{ml min}^{-1} \text{kg}^{-1}$).

Statistical analyses The primary endpoint of the study was change in $\text{AUC}_{\text{glucose}0-120 \text{ min}}$ (response/non-response to lifestyle intervention). Data are given as mean $\pm$ SE. A Wilcoxon-signed-rank test was used to compare variables before and after lifestyle intervention and during long-term follow-up. Prior to multivariable regression analyses, variable distributions were tested for normality using the Shapiro–Wilk test. Non-normally distributed variables were transformed to their natural logarithm. To identify key model determinants, forward stepwise multivariable linear regression analyses were performed. A p value ≤ 0.05 was considered statistically significant. The software package JMP 11 (SAS Institute, Cary, NC, USA) was used for all other analyses.

Results

Longitudinal results A total of 413 participants were enrolled in the study, with 357 undertaking 9 months of intensive lifestyle intervention, and 300 completing the full intervention in the 24 month study after (Fig. 1). We invited these 300 participants, via written invitation or telephone call, to a

Fig. 1 Trial profile of the TULIP study: screening, enrolment and post-intervention follow-up period (8.7 ± 1.6 years)

long-term follow-up visit; 190 individuals (63.3%) attended after a mean total follow-up time (time between baseline and long-term follow-up) of 8.7 ± 1.6 years (Fig. 1). The main reason for not participating in the long-term follow-up was unknown address or loss to follow-up (Fig. 1). Table 1 shows the participant characteristics during the four visits. After 9 months of intervention, AUCglucose_{0–120 min} values improved in 63% of the participants (responders). This ratio of responders was decreased at the long-term follow-up to 43%. At the long-term follow-up, 46% of the 190 participants had a normal glucose regulation (normal glucose tolerance and

normal fasting glucose), 43% participants had IFG and/or IGT and 21 participants (11%) developed diabetes (Fig. 2).

Among the 190 participants who attended all visits, fasting and postprandial plasma glucose levels, insulin sensitivity, the disposition index, fasting proinsulin, fasting insulin and fasting PI/I ratio improved during the 9 month intensive period of the lifestyle intervention. Body weight, liver fat content, TAT, SCAT and VAT decreased (Table 2, Fig. 3).

Comparing the baseline with the long-term follow-up variables after 9 years, fasting PI/I ratio and fasting proinsulin improved from baseline to long-term follow-up (both

Table 1 Characteristics of participants at different time points

Characteristic	Baseline	Lifestyle intervention		Long-term follow-up
		9 months	24 months	
<i>n</i>	413	357	300	190
Sex (F/M)	258/155	222/135	182/118	112/78
Age (years)	45 ± 11	47 ± 11	49 ± 11	58 ± 10
Body weight (kg)	88.9 ± 1.0	85.5 ± 1.0	86.0 ± 1.1	86.9 ± 1.4
BMI (kg/m ²)	30.3 ± 0.3	29.2 ± 0.3	29.3 ± 0.3	30.1 ± 0.4
WHR	0.89 ± 0.0	0.88 ± 0.0	0.89 ± 0.0	0.91 ± 0.01
Fasting blood glucose (mmol/l)	5.2 ± 0.0	5.2 ± 0.0	5.3 ± 0.0	5.7 ± 0.1
2 h blood glucose (mmol/l)	6.9 ± 0.1	6.7 ± 0.1	7.2 ± 0.1	7.2 ± 0.2
AUCglucose _{0–120 min} (mmol × h/l)	16.1 ± 0.1	15.6 ± 0.2	16.4 ± 0.2	16.9 ± 0.3

Data are means $\pm$ SE

F, female; M, male

Fig. 2 Progression of glycaemic status from baseline to long-term follow-up. Data are given as percentages. $n = 190$ participants. Black, baseline; light grey, 9 months of lifestyle intervention; dark grey, 24 months of lifestyle intervention; white, long-term follow-up. NGR, normal glucose regulation; T2DM, type 2 diabetes mellitus

$p \leq 0.0001$), while post-challenge (2 h) plasma glucose, $\text{AUCglucose}_{0-120 \text{ min}}$ during the OGTT and liver fat content did not change significantly. In contrast, fasting glucose levels increased and insulin sensitivity, as well as the disposition index, decreased significantly. Furthermore, body weight, TAT, SCAT, VAT and fasting insulin increased with the concomitant deterioration of cardiorespiratory fitness during this period (all $p \leq 0.05$, Table 2, Fig. 3).

Impact of body weight change during the first 9 months of lifestyle intervention on long-term success Decrease in body weight during the lifestyle intervention was found to associate with the success of the lifestyle intervention to improve glycaemia [3, 4, 16]. We therefore performed multivariable linear regression analysis with change in BMI during the intensive period of the lifestyle intervention as effect variable and $\text{AUCglucose}_{0-120 \text{ min}}$ levels at long-term follow-up as the dependent variable. The model was additionally adjusted for sex, age, follow-up time, $\text{AUCglucose}_{0-120 \text{ min}}$ and BMI at baseline. The change in BMI during the intensive period of the lifestyle intervention was associated with the $\text{AUCglucose}_{0-120 \text{ min}}$ level in long-term follow-up ($\beta = 0.206$, $p = 0.0006$; Table 3, model 1).

Impact of insulin sensitivity and insulin secretion on long-term success of the lifestyle intervention Insulin secretion and insulin sensitivity are important pathophysiological factors in the pathogenesis of type 2 diabetes [11]. Therefore, these variables and the change of both during the intensive phase of lifestyle intervention were added to model 1. As shown in Table 3 (model 2), the baseline insulin secretion ($\beta = -0.436$, $p \leq 0.0001$) and insulin sensitivity ($\beta = -0.405$, $p = 0.0009$) and the change in insulin sensitivity ($\beta = -0.230$, $p = 0.0015$), were associated with the $\text{AUCglucose}_{0-120 \text{ min}}$ level at the long-term follow-up.

To visualise the effect of baseline insulin secretion on the cumulative probability of not developing diabetes, Kaplan–

Meier survival curves were generated. Not a single diabetes case was diagnosed in individuals with a low disposition index compared with a high disposition index at baseline (Fig. 4a).

Impact of hepatic lipid content on the long-term success of the lifestyle intervention In a next step, hepatic lipid content at baseline and change in content during the intensive phase of the lifestyle intervention were added to the model (Table 3, model 3). Hepatic lipid content at baseline ($\beta = 0.231$, $p = 0.0207$) predicted $\text{AUCglucose}_{0-120 \text{ min}}$ at long-term follow-up, in addition to baseline insulin secretion, change in insulin sensitivity and age.

To visualise the effect of baseline hepatic lipid content on the cumulative probability of not developing diabetes, Kaplan–Meier survival curves were generated. In participants with or without NAFLD, individuals without NAFLD at baseline had a significantly lower risk for developing diabetes compared with individuals with NAFLD (logrank test, $p = 0.0094$) (Fig. 4b).

Impact of fasting proinsulin to insulin conversion on the long-term success of the lifestyle intervention Proinsulin conversion has been shown to have an impact on diabetes risk [34, 35]. Therefore, the fasting PI/I ratio at baseline and the change in the fasting PI/I ratio during the intensive phase of the lifestyle intervention were added to model 3. The baseline fasting PI/I ratio predicted the long-term $\text{AUCglucose}_{0-120 \text{ min}}$ ($\beta = 0.204$, $p = 0.0444$), in addition to the baseline insulin secretion and the change in insulin sensitivity during the intensive phase of the lifestyle intervention. There is an inverse correlation of fasting PI/I ratio and hepatic lipid content ($\beta = -0.208$, $p = 0.0213$), thus, the impact of liver fat was no longer significant in this model. Of note, model 4 gave the highest r^2 value for glucose levels at long-term follow-up, explaining 59% of variability in $\text{AUCglucose}_{0-120 \text{ min}}$. Furthermore, the ratio of proinsulin to C-peptide impacts similarly on long-term glycaemia as the PI/I ratio (see electronic supplementary material [ESM] Table 1).

Discussion

This long-term follow-up analysis of a standard lifestyle intervention programme aiming at preventing type 2 diabetes provides three major results: first, we show, for the first time, data on the long-term development of body fat composition and ectopic fat deposition in the liver, together with changes in insulin sensitivity and insulin secretion following a lifestyle intervention. Second, we show that the long-term success in maintaining glycaemia can be inferred from baseline variables and short-term response to the lifestyle intervention. Third, we identified a baseline high-risk phenotype for the long-term glycaemic non-response that includes, besides impaired

Table 2 Anthropometric and metabolic profile of 190 participants from baseline to the long-term follow-up

Characteristic	Baseline	Lifestyle intervention		Long-term follow-up	<i>p</i> value		
		9 months	24 months		Baseline vs 9 months' intervention	Baseline vs 24 months' intervention	Baseline vs long-term follow-up
Sex (f/m)	112/78						
Age (years)	49 ± 10	49 ± 10	51 ± 10	58 ± 10			
Body weight (kg)	86.3 ± 1.3	83.3 ± 1.2	84.6 ± 1.3	86.9 ± 1.4	< 0.0001	< 0.0001	0.3917
Body height (cm)	170.4 ± 0.7	170.5 ± 0.7	170.4 ± 0.7	169.7 ± 0.7	0.2500	0.5093	< 0.0001
BMI (kg/m ²)	29.7 ± 0.4	28.6 ± 0.4	29.1 ± 0.4	30.1 ± 0.4	< 0.0001	0.0001	0.0211
WHR	0.9 ± 0.01	0.89 ± 0.01	0.89 ± 0.01	0.91 ± 0.01	< 0.0001	0.0115	0.0043
Fasting blood glucose (mmol/l)	5.3 ± 0.0	5.2 ± 0.0	5.3 ± 0.0	5.7 ± 0.1	0.0171	0.5365	< 0.0001
2 h blood glucose (mmol/l)	7.1 ± 0.1	6.8 ± 0.1	7.3 ± 0.1	7.2 ± 0.2	0.0136	0.5654	0.4441
AUCglucose _{0–120 min} (mmol × h/l)	16.6 ± 0.2	15.9 ± 0.2	16.6 ± 0.2	16.9 ± 0.3	0.0004	0.9425	0.1771
Fasting insulin (pmol/l)	65.21 ± 3.38	55.30 ± 2.72	61.46 ± 2.78	101.95 ± 4.44	0.0006	0.1806	< 0.0001
Fasting proinsulin (pmol/l)	5.86 ± 0.56	4.02 ± 0.45	3.42 ± 0.26	3.24 ± 0.21	0.0020	0.0001	< 0.0001
Fasting proinsulin conversion (PI/I ratio) ^a	0.113 ± 0.01	0.085 ± 0.01	0.071 ± 0.01	0.038 ± 0.00	0.0139	0.0208	< 0.0001
Fasting C-peptide (pmol/l)	613 ± 20	553 ± 16	583 ± 19	616 ± 18	< 0.0001	0.0567	0.8946
Fasting proinsulin to C-peptide ratio	0.011 ± 0.001	0.008 ± 0.011	0.006 ± 0.001	0.007 ± 0.001	0.0089	0.0123	0.0002
Insulin sensitivity	12.1 ± 0.5	13.8 ± 0.6	12.6 ± 0.5	8.4 ± 0.4	0.0001	0.7301	0.0001
IGI _{0–30 min}	133 ± 8	134 ± 8	136 ± 10	163 ± 8	0.3948	0.3741	< 0.0001
Insulin secretion (disposition index)	1411 ± 113	1655 ± 126	1458 ± 132	1169 ± 77	0.0001	0.0836	< 0.0001
$\dot{V}O_{2max}$ (ml min ⁻¹ kg ⁻¹) ^b	26.5 ± 0.7	27.2 ± 0.7	26.4 ± 0.8	21.0 ± 0.6	0.0548	0.8802	< 0.0001
Hepatic lipid content (%) ^c	6.0 ± 0.7	4.1 ± 0.4	4.8 ± 0.5	6.1 ± 0.6	< 0.0001	0.0031	0.5452
TAT (l) ^d	30.4 ± 1.1	27.9 ± 1.0	29.2 ± 1.1	34.9 ± 1.1	< 0.0001	0.0105	< 0.0001
VAT (l) ^e	3.5 ± 0.2	2.9 ± 0.2	3.2 ± 0.2	4.8 ± 0.3	< 0.0001	0.0003	< 0.0001
SCAT (l) ^f	9.0 ± 0.5	7.9 ± 0.4	6.8 ± 0.4	12.6 ± 0.6	< 0.0001	< 0.0001	< 0.0001

Data are means ± SE; data were available for the following numbers of participants: ^a 151; ^b 100; ^c 99; ^d 104; ^e 106; ^f 93

Liver fat content was measured by ¹H-MRS. A Wilcoxon-signed-rank test was used to compare variables before and after lifestyle intervention and at long-term follow-up

insulin secretion and insulin sensitivity, fatty liver or impaired fasting proinsulin conversion.

Recent studies have shown that a lifestyle intervention is effective in preventing or delaying the development of type 2 diabetes during both short- and long-term follow-up [3–7, 45, 46]. The lifestyle changes comprised several beneficial changes in diet, as well as increased physical activity, with beneficial effects on blood glucose levels and a significant reduction in diabetes incidence [3, 45]. The TULIP intervention was most intensive during the first 9 months. Consequently, the benefit was most prominent during this time. Body weight reduction during the intensive period was moderate, as observed in two other studies including individuals with IGT [3, 45].

Using whole-body MRI, we observed a marked reduction in major body fat compartments (TAT, VAT and SCAT) between 10% and 15% during the first 9 months of intensive

intervention. Importantly, liver fat content improved by more than 30% during that period. This ‘qualified’ weight reduction with decreases in visceral fat mass and liver fat content improved insulin sensitivity by nearly 15%. These risk factors are known to be associated with metabolically unhealthy obesity [47]. VAT and intrahepatic fat are the source of several hormones and cytokines that act against insulin and initiate a chronic systemic activation of the immune system. This chronic systemic inflammation is the key driver for further impairment of insulin resistance and also for the chronic progressive nature of the disease [48]. Lifestyle interventions can only modulate disease development if there is an impact on the hormonal activity of the body fat compartments and not just on the volume of body fat [49].

In contrast to the favourable short-term effects of a lifestyle intervention, the improvements in metabolic variables are lost over time when body weight and body fat composition, as

Fig. 3 Progression of metabolic variables and fat compartments from baseline to the long-term follow-up. Data are given as mean ± SE. AUCglucose_{0–120 min} during OGTT (a), ISI (b), insulin secretion (disposition index [ISI × IGI_{0–30 min}]) (c), liver fat content (d), VAT (e) and fasting PI/I ratio (f) at baseline, after 9 months and 24 months of lifestyle intervention and at long-term follow-up; LT, long-term follow-up

well as insulin sensitivity and insulin secretion, deteriorate. Despite gain in body weight and body fat, postprandial blood glucose levels and liver fat content were comparable with baseline levels, even after 9 years (Table 2, Fig. 3). One important variable that improved during the lifestyle intervention and was improved at long-term follow-up was fasting proinsulin conversion. As fasting insulin was doubled at long-term follow-up compared with baseline and C-peptide was unchanged, this indicates declining hepatic insulin clearance over this time (9 years), suggesting improved proinsulin conversion. Fasting proinsulin levels also decreased by ~ 50%, also indicating that proinsulin conversion was improved.

In addition, it should be highlighted that a rate of 11% incident diabetes is rather low compared with similar lifestyle intervention programmes. In the lifestyle intervention group of the Finnish DPS and the US DPPOS, 29% and 40% of participants, respectively, developed diabetes during the long-term follow-up [4, 7]. This might in part be because both studies exclusively included participants with IGT at baseline, while the TULIP study included a metabolically healthier cohort. Taken together, our results are in line with earlier studies showing that healthy changes in diet and increased exercise can have beneficial long-

term effects on glycaemia, and offer encouraging evidence for the effectiveness of a lifestyle intervention, even without a large reduction in body weight [4, 6, 7, 20].

Nevertheless, the problem of non-response with respect to an improvement of glycaemia during the lifestyle intervention in a substantial number of participants remains [4, 6, 7, 20]. It is, therefore, clinically important to define risk factors and risk phenotypes associated with such a non-response.

One factor might be the decrease in insulin sensitivity that is correlated with the amount of weight loss [19]. However, the correlation of weight loss and glycaemia is not very strong [15]. Paradoxically, in the US DPPOS a stronger weight loss even adversely affected diabetes risk, independent of the previous intervention [20]. Thus, other metabolic determinants may additionally affect the long-term effectiveness of a lifestyle intervention. In the present study, we addressed the pathophysiological determinants that predict non-response to the lifestyle intervention. We were able to show that low insulin secretion capacity at baseline, low insulin sensitivity and impaired improvement in insulin sensitivity during the intensive first 9 months of the lifestyle intervention predicted non-response to the intervention. These findings accord with previous studies that have shown that both baseline insulin secretion and insulin sensitivity, as well as the change thereof during a lifestyle intervention, are predictors of diabetes development [16, 19, 50] or, vice versa, regression to normal glucose tolerance [20, 21].

A new finding was that a high liver fat content at baseline or an impaired fasting relative proinsulin to insulin conversion at baseline were important determinants of impaired glycaemia at long-term follow-up, independent of insulin secretion and insulin sensitivity. Models containing these pathophysiological factors provided a better prediction ($r^2 = 0.55$ and 0.59 , respectively) of long-term glycaemia than a simpler model containing baseline BMI as well as change in BMI ($r^2 = 0.45$) (Table 3).

The conversion of proinsulin to insulin is thought to be a biomarker for beta cell function [26, 51, 52]. It is important that in the present study proinsulin to insulin conversion predicted long-term glycaemia after the lifestyle intervention, independently of insulin secretion and insulin sensitivity (model 4). Our results are in line with earlier studies that showed that increased proinsulin levels, which are the consequence of impaired proinsulin conversion to insulin, predict worsening of glucose tolerance status [53, 54]. Furthermore, it has been shown that fasting proinsulin conversion and the acute insulin response independently impact on the development of type 2 diabetes [27, 55] highlighting that there are multiple aspects to beta cell dysfunction. In this context, the improvement of proinsulin to insulin conversion after the lifestyle intervention and at the long-term follow-up is an important finding. One may speculate that proinsulin conversion is a useful biomarker for both the prediction of diabetes risk and monitoring the success of the lifestyle intervention.

Table 3 Prediction of the long-term success of a lifestyle intervention

Characteristic	Model 1, $n = 184$, $r^2 = 0.45, p < 0.0001^a$				Model 2, $n = 184$, $r^2 = 0.52, p < 0.0001^a$				Model 3, $n = 127$, $r^2 = 0.55, p < 0.0001^a$				Model 4, $n = 109$, $r^2 = 0.59, p < 0.0001^a$			
	Std β	SE	t ratio	p	Std β	SE	t ratio	p	Std β	SE	t ratio	p	Std β	SE	t ratio	p
Age (years)	0.108	0.059	1.74	0.0838	0.112	0.056	1.90	0.0597	0.141	0.067	1.99	0.0494	0.143	0.068	1.94	0.0557
Sex (f/m)	-0.062	0.012	-1.11	0.2672	-0.144	0.012	-2.57	0.0109	-0.099	0.015	-1.42	0.1587	-0.061	0.015	-0.83	0.4100
Follow-up (years)	-0.001	0.057	-0.01	0.9896	0.016	0.054	0.31	0.7575	-0.024	0.061	-0.35	0.7244	-0.045	0.061	-0.62	0.5391
AUCglucose during OGTT (mmol $\times$ h/l) baseline	0.586	0.076	9.22	< 0.0001	0.245	0.139	2.10	0.0369	0.207	0.172	1.51	0.1337	0.336	0.182	2.41	0.0177
BMI baseline (kg/m 2)	0.169	0.072	2.85	0.0049	0.151	0.081	2.26	0.0252	0.085	0.106	1.02	0.3087	0.088	0.112	0.97	0.3354
Change in BMI 0–9 months' lifestyle intervention (kg/m 2)	0.206	0.219	3.52	0.0006	0.105	0.237	1.65	0.1017	0.127	0.301	1.60	0.1132	0.117	0.318	1.40	0.1648
ISI at baseline																
Change in ISI 0–9 months' lifestyle intervention					-0.405	0.044	-3.37	0.0009	-0.286	0.051	-1.96	0.0529	-0.321	0.056	-1.98	0.0506
IGI $_{0-30 \text{ min}}$ at baseline					-0.230	0.034	-3.22	0.0015	-0.186	0.042	-2.05	0.0422	-0.220	0.046	-2.22	0.0286
Change in IGI $_{0-30 \text{ min}}$ 0–9 months' lifestyle intervention					-0.436	0.035	-4.01	< 0.0001	-0.446	0.041	-3.43	0.0008	-0.387	0.044	-2.80	0.0062
Hepatic lipid content at baseline (%)					-0.061	0.026	-1.05	0.2932	-0.064	0.029	-0.91	0.3625	-0.070	0.030	-0.94	0.3485
Change in hepatic lipid content 0–9 months' lifestyle intervention (%)									0.231	0.019	2.35	0.0207	0.145	0.019	1.43	0.1570
Fasting proinsulin conversion (PI/I) at baseline									0.0553	0.024	0.65	0.5138	0.110	0.025	1.26	0.2108
Fasting proinsulin conversion (PI/I) 0–9 months' lifestyle intervention													0.204	0.020	2.04	0.0444
													0.105	0.018	1.11	0.2689

Models 1 to 4 are multivariable regression models, with AUCglucose $_{0-120 \text{ min}}$ as the dependent variable

Change is fold change during intensive phase of lifestyle intervention

The variance inflation factors (VIFs) are in the range 1–5, indicating a minor or moderate collinearity

Std, standard

^a p value for the whole model

Fig. 4 Cumulative probability of not developing type 2 diabetes and prediction models. **(a)** Cumulative probability of not developing type 2 diabetes in the TULIP study in a group with a high (dashed line) or low (solid line) disposition index at baseline, according to whether they were

above or below the median. Logrank test, $p < 0.0001$. **(b)** Cumulative probability of not developing type 2 diabetes in the TULIP study in a group with (solid line) or without NAFLD (dashed line) at baseline (cut-off point, 5.56% liver fat). Logrank test, $p = 0.0094$

For the design, development and practice of improved and more successful lifestyle intervention programmes, it is important to reveal risk factors for short- and long-term success of a lifestyle intervention. Regarding short-term success, we previously found that the phenotype encompassing participants with a low disposition index or insulin-resistant NAFLD have a high risk for acute glycaemic non-response to lifestyle intervention [15]. The present study now demonstrates that such a phenotype is also associated with long-term non-response and extends the phenotype to impaired proinsulin conversion. Individuals with such a high-risk phenotype may be put on a more intensive lifestyle intervention at an earlier stage, or even receive pharmacological therapy.

One limitation of the present analysis is that the TULIP study was designed as an uncontrolled study. Therefore, we cannot compare the risk of non-response to the lifestyle intervention (i.e. improvement in glycaemia) with the risk of developing diabetes without intervention. However, a lifestyle intervention is standard care in individuals at high risk for type 2 diabetes [56] after the publication of the landmark studies, the DPS and DPP, and thus, a non-treated control group seems inappropriate. Furthermore, our study was conducted at a single academic research centre and the results cannot probably be generalised to other populations.

In conclusion, we found that, independent of the important change in body weight, an impaired insulin secretion, a fatty liver or an impaired fasting proinsulin conversion before lifestyle intervention predicted glycaemic non-response to the lifestyle intervention programme. Our current results may help to develop individualised prevention programmes targeting those with at-risk phenotypes. Furthermore, they may also aid monitoring during such programmes and may help to identify those for whom treatment intensification will be necessary.

Acknowledgements We thank all the study participants for their cooperation with this project. We gratefully acknowledge the excellent technical assistance of A. Bury, E. Kollmar, A. Vosseler and A. Dessecker

(all University of Tübingen, Germany). We further thank J. Machann and F. Schick from the Section for Experimental Radiology for the excellent body imaging and cooperation within the study.

Data availability The datasets generated during and/or analysed during the current study are not publicly available because the participants have not granted permission for this. The datasets are available from the corresponding author on reasonable request.

Funding The study was supported in part by grants from the Deutsche Forschungsgemeinschaft and the German Federal Ministry of Education and Research (BMBF) to the German Centre for Diabetes Research (DZD). The study was supported by a grant of the DZD to AF.

Duality of interest The study was initiated by the authors and there are no other sponsors other than those acknowledged. The authors declare that there is no duality of interest associated with this manuscript.

Contribution statement All authors provided substantial contributions to the conception and design, acquisition of data or analysis and interpretation of data, drafting the article or revising it critically for important intellectual content. They all gave final approval of the version to be published. The most important contributions of each author were as follows. VS and AF researched data and wrote the manuscript. RW, CS, LF, KK, AP and MH researched data and edited the manuscript. AF, NS and HUH designed the study, contributed to the discussion and reviewed the manuscript. AF is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Tuomilehto J, Lindström J, Eriksson JG et al (2001) Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N Engl J Med* 344:1343–1350
2. Lindström J, Ilanne-Parikka P, Peltonen M et al (2006) Sustained reduction in the incidence of type 2 diabetes by lifestyle intervention: follow-up of the Finnish Diabetes Prevention Study. *Lancet* 368:1673–1679
3. Knowler WC, Barrett-Connor E, Fowler SE et al (2002) Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med* 346:393–403

4. Diabetes Prevention Program Research Group, Knowler WC, Fowler SE et al (2009) 10-year follow-up of diabetes incidence and weight loss in the Diabetes Prevention Program Outcomes Study. *Lancet* 374:1677–1686
5. Pan XR, Li GW, Hu YH et al (1997) Effects of diet and exercise in preventing NIDDM in people with impaired glucose tolerance. The Da Qing IGT and Diabetes Study. *Diabetes Care* 20:537–544
6. Li G, Zhang P, Wang J et al (2008) 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* 371:1783–1789
7. Lindström J, Peltonen M, Eriksson JG et al (2013) Improved lifestyle and decreased diabetes risk over 13 years: long-term follow-up of the randomised Finnish Diabetes Prevention Study (DPS). *Diabetologia* 56:284–293
8. Ramachandran A, Snehalatha C, Mary S et al (2006) 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* 49:289–297
9. Schwarz PE, Greaves CJ, Lindström J et al (2012) Nonpharmacological interventions for the prevention of type 2 diabetes mellitus. *Nat Rev Endocrinol* 8:363–373
10. Kantartzis K, Thamer C, Peter A et al (2009) High cardiorespiratory fitness is an independent predictor of the reduction in liver fat during a lifestyle intervention in non-alcoholic fatty liver disease. *Gut* 58:1281–1288
11. Häring H-U (2016) Novel phenotypes of prediabetes? *Diabetologia* 59:1806–1818
12. Ferrannini E (2014) Definition of intervention points in prediabetes. *Lancet Diabetes Endocrinol* 2:667–675
13. Kahn SE, Cooper ME, Del Prato S (2014) Pathophysiology and treatment of type 2 diabetes: perspectives on the past, present, and future. *Lancet* 383:1068–1083
14. Stefan N, Fritsche A, Schick F, Häring H-U (2016) Phenotypes of prediabetes and stratification of cardiometabolic risk. *Lancet Diabetes Endocrinol* 4:789–798
15. Stefan N, Staiger H, Wagner R et al (2015) A high-risk phenotype associates with reduced improvement in glycaemia during a lifestyle intervention in prediabetes. *Diabetologia* 58:2877–2884
16. Kitabchi AE, Tempresa M, Knowler WC et al (2005) 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* 54:2404–2414
17. Abdul-Ghani MA, Williams K, DeFronzo RA, Stern M (2007) What is the best predictor of future type 2 diabetes? *Diabetes Care* 30:1544–1548
18. Lyssenko V, Almgren P, Anevski D et al (2005) Predictors of and longitudinal changes in insulin sensitivity and secretion preceding onset of type 2 diabetes. *Diabetes* 54:166–174
19. de Mello VDF, Lindström J, Eriksson J et al (2012) 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* 35:211–217
20. Perreault L, Pan Q, Mather KJ et al (2012) Effect of regression from prediabetes to normal glucose regulation on long-term reduction in diabetes risk: results from the Diabetes Prevention Program Outcomes Study. *Lancet* 379:2243–2251
21. Perreault L, Kahn SE, Christophi CA et al (2009) Regression from pre-diabetes to normal glucose regulation in the diabetes prevention program. *Diabetes Care* 32:1583–1588
22. Wareham NJ, Byrne CD, Williams R et al (1999) Fasting proinsulin concentrations predict the development of type 2 diabetes. *Diabetes Care* 22:262–270
23. Pfützner A, Kunt T, Hohberg C et al (2004) Fasting intact Proinsulin is a highly specific predictor of insulin resistance in type 2 diabetes. *Diabetes Care* 27:682–687
24. Mykkanen L, Zaccaro DJ, Hales CN et al (1999) The relation of proinsulin and insulin to insulin sensitivity and acute insulin response in subjects with newly diagnosed type II diabetes: the Insulin Resistance Atherosclerosis Study. *Diabetologia* 42:1060–1066
25. Bergman RN, Finegood DT, Kahn SE (2002) The evolution of β -cell dysfunction and insulin resistance in type 2 diabetes. *Eur J Clin Invest* 32:35–45
26. Kahn SE (2000) The importance of the beta-cell in the pathogenesis of type 2 diabetes mellitus. *Am J Med* 108(Suppl 6a):2S–8S
27. Hanley AJG, D’Agostino R, Wagenknecht LE et al (2002) Increased proinsulin levels and decreased acute insulin response independently predict the incidence of type 2 diabetes in the Insulin Resistance Atherosclerosis Study. *Diabetes* 51:1263–1270
28. Loopstra-Masters RC, Haffner SM, Lorenzo C et al (2011) Proinsulin-to-C-peptide ratio versus proinsulin-to-insulin ratio in the prediction of incident diabetes: the Insulin Resistance Atherosclerosis Study (IRAS). *Diabetologia* 54:3047–3054
29. Zethelius B, Byberg L, Hales CN et al (2003) Proinsulin and acute insulin response independently predict type 2 diabetes mellitus in men—report from 27 years of follow-up study. *Diabetologia* 46:20–26
30. Pfützner A, Pfützner AH, Larbig M, Forst T (2004) Role of intact Proinsulin in diagnosis and treatment of type 2 diabetes mellitus. *Diabetes Technol Ther* 6:405–412
31. Uusitupa M, Lindi V, Louheranta A et al (2003) Long-term improvement in insulin sensitivity by changing lifestyles of people with impaired glucose tolerance: 4-year results from the Finnish Diabetes Prevention Study. *Diabetes* 52:2532–2538
32. Yoshioka N, Kuzuya T, Matsuda A, Iwamoto Y (1989) Effects of dietary treatment on serum insulin and Proinsulin response in newly diagnosed NIDDM. *Diabetes* 38:262–266
33. Kahn SE, Halban PA (1997) Release of incompletely processed proinsulin is the cause of the disproportionate proinsulinemia of NIDDM. *Diabetes* 46:1725–1732
34. Røder ME, Porte D, Schwartz RS, Kahn SE (1998) Disproportionately elevated proinsulin levels reflect the degree of impaired B cell secretory capacity in patients with noninsulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* 83:604–608
35. Larsson H, Ahrén B (1999) Relative Hyperproinsulinemia as a sign of islet dysfunction in women with impaired glucose tolerance. *J Clin Endocrinol Metab* 84:2068–2074
36. Hofso D, Jenssen T, Bollerslev J et al (2011) Beta cell function after weight loss: a clinical trial comparing gastric bypass surgery and intensive lifestyle intervention. *Eur J Endocrinol* 164:231–238
37. Ramos JS, Dalleck LC, Borrani F et al (2016) The effect of different volumes of high-intensity interval training on proinsulin in participants with the metabolic syndrome: a randomised trial. *Diabetologia* 59:2308–2320
38. McCaffery JM, Jablonski KA, Franks PW et al (2011) TCF7L2 polymorphism, weight loss and proinsulin : insulin ratio in the diabetes prevention program. *PLoS One* 6:e21518
39. Kantartzis K, Machann J, Schick F et al (2011) Effects of a lifestyle intervention in metabolically benign and malign obesity. *Diabetologia* 54:864–868
40. Machann J, Thamer C, Stefan N et al (2010) Follow-up whole-body assessment of adipose tissue compartments during a lifestyle intervention in a large cohort at increased risk for type 2 diabetes. *Radiology* 257:353–363
41. Haupt A, Thamer C, Heni M et al (2010) Gene variants of TCF7L2 influence weight loss and body composition during lifestyle intervention in a population at risk for type 2 diabetes. *Diabetes* 59:747–750
42. Pfützner A, Kunt T, Langenfeld M et al (2005) Clinical and laboratory evaluation of specific chemiluminescence assays for intact and total proinsulin. *Clin Chem Lab Med* 41:1234–1238
43. Matsuda M, DeFronzo RA (1999) Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care* 22:1462–1470

44. Szczepaniak LS, Nurenberg P, Leonard D et al (2005) Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. *Am J Physiol Endocrinol Metab* 288:E462–E468
45. Lindström J, Louheranta A, Mannelin M et al (2003) The Finnish Diabetes Prevention Study (DPS): lifestyle intervention and 3-year results on diet and physical activity. *Diabetes Care* 26:3230–3236
46. Schellenberg ES, Dryden DM, Vandermeer B et al (2013) Lifestyle interventions for patients with and at risk for type 2 diabetes: a systematic review and meta-analysis. *Ann Intern Med* 159:543–551
47. Stefan N, Kantartzis K, Machann J et al (2008) Identification and characterization of metabolically benign obesity in humans. *Arch Intern Med* 168:1609–1616
48. Hotamisligil GS (2006) Inflammation and metabolic disorders. *Nature* 444:860–867
49. Stefan N, Hennige AM, Staiger H et al (2006) α_2 -Heremans-Schmid glycoprotein/fetuin-A is associated with insulin resistance and fat accumulation in the liver in humans. *Diabetes Care* 29:853–857
50. Snehalatha C, Mary S, Selvam S et al (2009) Changes in insulin secretion and insulin sensitivity in relation to the glycemic outcomes in subjects with impaired glucose tolerance in the Indian Diabetes Prevention Programme-1 (IDPP-1). *Diabetes Care* 32: 1796–1801
51. Ahren B (2005) Type 2 diabetes, insulin secretion and beta-cell mass. *Curr Mol Med* 5:275–286
52. Fritsche A, Madaus A, Stefan N et al (2002) Relationships among age, Proinsulin conversion, and β -cell function in nondiabetic humans. *Diabetes* 51:S234–S239
53. Vangipurapu J, Stančáková A, Kuulasmaa T et al (2015) Both fasting and glucose-stimulated proinsulin levels predict hyperglycemia and incident type 2 diabetes: a population-based study of 9,396 Finnish men. *PLoS One* 10:e0124028
54. Nijpels G, Popp-Snijders C, Kostense PJ et al (1996) Fasting proinsulin and 2-h post-load glucose levels predict the conversion to NIDDM in subjects with impaired glucose tolerance: the Hoorn study. *Diabetologia* 39:113–118
55. Haffner SM, Mykkanen L, Valdez RA et al (1994) Disproportionately increased proinsulin levels are associated with the insulin resistance syndrome. *J Clin Endocrinol Metab* 79:1806–1810
56. American Diabetes Association (2016) Prevention or delay of type 2 diabetes. *Diabetes Care* 39(Suppl 1):S36–S38