The diabetes risk phenotype of young women with recent gestational diabetes

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Context: The pathogenesis of type 2 diabetes (T2D) is still incompletely understood. In-depth phenotyping of young individuals at risk for T2D can contribute to the understanding of this process.

Objective: To metabolically characterize women with recent gestational diabetes (GDM), an at-risk cohort for T2D.

Study participants: 147 consecutively recruited women 3–16 months after pregnancy, women who had GDM and women after a normoglycemic pregnancy (controls) in a 2:1 ratio

Design: Mono-center cross-sectional analysis (PPS-Diab study)

Methods: 5-point OGTT with calculation of insulin sensitivity (ISI) and disposition index (DI; validation by euglycemic clamp and IVGTT), anthropometrics, medical and family history, clinical chemistry and biomarkers, statistical modelling, MRI/MRS substudy (body fat distribution, liver and muscle fat; n=66)

Results: Compared to control subjects, women post GDM had a reduced DI, higher levels of plasma fetuin-A and a lower ISI. A low ISI was also the major determinant of pathologic glucose tolerance after GDM. The factors most strongly predictive of low insulin sensitivity were high plasma leptin, BMI, triglycerides, and waist circumference. Ectopic lipids showed no BMI-independent associations with having had GDM or low insulin sensitivity in an MRI substudy.

Conclusions: We found that beta cell function is already impaired in women with recent GDM, a young at-risk cohort for T2D. Additionally, our data suggest that fetuin-A and leptin signaling may be important early contributors to the pathogenesis of T2D, at this disease stage equally or more relevant than ectopic lipids and low-grade inflammation.

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Abbreviations:

Type 2 diabetes (T2D) develops chronically over years to decades before it becomes apparent. Many secondary changes in metabolism, hormonal signaling and body composition occur during that time, which are difficult to discriminate from those that initially started the pathophysiological process. For that reason the early pathogenesis of T2D is still incompletely understood. Certainly a combination of insulin resistance (IR) and inadequately low insulin secretion is necessary for T2D to finally manifest itself (1) but when and how these metabolic alterations come about and how they interact is unclear. Obesity contributes to the pathophysiology in many cases but only a fraction of obese individuals develops diabetes (2). Therefore specific links between obesity and IR must exist. Ectopic lipid depositions and subclinical inflammation are currently considered the most likely candidates for this connection (3, 4). However, other pathophysiological models of IR have been proposed (5) and low insulin sensitivity has also been observed in the absence of ectopic organ triglycerides (6). The beta cell dysfunction of T2D on the other hand seems to have a relevant, however not fully explanatory genetic component (7) and is often considered as a late event in the pathogenetic process (3).

Metabolic phenotyping of young at-risk subjects has contributed greatly to our understanding of the T2D pathogenesis (8). Because no clear-cut biomarkers exist to identify these individuals, such studies have mostly relied on prospective, population based designs with a long follow up or on a positive family history. The first approach requires large sample sizes and is restrictive with respect to the depth of phenotyping. Relying on family history circumvents these problems but only looks at a selected scenario. Additionally, despite the known heritability of T2D, the capacity to predict a person's individual diabetes risk based on family history alone is limited (9).

We followed a third approach to identify young subjects with a high risk of T2D and examined women from the general population with recent gestational diabetes (GDM). GDM affects 5%–10% of pregnant women and is associated with an about 10-fold increased risk for subsequent T2D (10). Women after a normoglycemic pregnancy served as control subjects in our study. The aims of this study were to describe the differences in the metabolic phenotype and in body composition between women with a recent history of GDM and controls and to characterize metabolic factor associated with the persistence of pathologic glucose tolerance after GDM.

Materials and Methods

Study population

Individuals included in the present analysis were participants of the prospective, mono-center observational study PPS-Diab ("Prediction, Prevention and Subclassification of type 2 diabetes") enrolled between November 2011 and December 2013. The study population consisted of women with GDM during their last pregnancy (post-GDM) and women following a normoglycemic pregnancy (controls) in the ratio 2:1. The cohorts were recruited consecutively from the Diabetes Center and the obstetrics department of the University Hospital (Klinikum der Universität München) in Munich, Germany. Eligible women were within 3 to 16 months after delivery. The diagnosis of GDM was based on a 75g oral glucose tolerance test (OGTT) after the 23rd week of gestation. The cut-off values for GDM were 92/180/153 mg/dl plasma glucose following the IADPSG recommendations. One woman had overt diabetes during pregnancy according to the IADPSG criteria (fasting plasma glucose $\geq$ 126 mg/dl). However, because she reverted to normoglycemia after pregnancy, she was not excluded from the analysis. Women could participate as controls if they had no history of GDM in any previous pregnancy and either a normal 75g OGTT (n = 46 women) or a normal 50g screening OGTT (<135 mg/dl plasma glucose, n = 5 women) after the 23rd week of gestation. Exclusion criteria for this study were alcohol or substance abuse and chronic diseases requiring medication (except for hypothyroidism (n = 23) and mild hypertension (n = 1)). Written informed consent was obtained from all study participants and the protocol was approved by the ethical review committee of the Ludwig-Maximilians-Universität. All data used in this analysis were collected at the baseline visit of the PPS-Diab study, 3 to 16 months after the index pregnancy.

Anthropometric and clinical measurements

Body weight and fat mass were measured using a bioelectrical impedance analysis (BIA) scale (Tanita BC-418, Tanita Corporation, Tokyo, Japan). A 5-point 75g OGTT was performed (see e-supplement for details of blood sample processing and laboratory analyses). Systolic and diastolic blood pressure (BP) readings were obtained from all subjects in a sitting position (both arms, repeated measurements, average from "higher" arm recorded). Impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and T2D were defined according to the definitions of the American Diabetes Association (11).

Measures of insulin sensitivity and secretion from the OGTT

The Matsuda Index of insulin sensitivity (ISI) was calculated as described previously (12). It was also validated in a substudy of PPS-Diab against hyperinsulinemic euglycemic clamps (methodology in the e-supplement, **e-Table 1, e-figure 1**). The disposition index (DI) was calculated from the OGTT as ISI * ΔI 30′ (13). The rise in serum insulin during the first 30 minutes of the OGTT (ΔI 30′) was used because its predictive capacity for first-phase secretion in the IVGTT was significantly better than that of the insulinogenic index (the ratio of the increment of insulin to that of plasma glucose during the first 30 minutes of the OGTT; IGI), which has been used in other studies to calculate the DI (**e-Table 2, e-figures 2, 3**).

Magnetic resonance imaging (MRI) and Magnetic resonance spectroscopy (MRS)

All study participants were invited for a whole body MRI study on a separate day. 66 study participants completed the

MRI/MRS examination (3 Tesla system; Ingenia or Achieva, Philips Healthcare, Best, The Netherlands). Participants were advised to refrain from heavy exercise 3 days before the MRI. We measured whole body, abdominal subcutaneous and abdominal visceral adipose tissue volume. Liver fat estimates were derived from a modified two-point Dixon sequence (14). Intramyocellular fat was determined in the anterior tibial and the soleus muscle by using a point resolved spectroscopy (PRESS; 15). See e-supplement for further details.

Statistical analyses

All metric and normally distributed variables are reported as mean±standard deviation; non-normally distributed variables as median [first quartile–third quartile]; categorical variables as frequency and percentage. Comparing groups, the Mann-Whitney-U test was used for metric variables and the χ^2 or Fisher's exact test for categorical variables. P-values < 0.05 were considered statistically significant. Univariate and multiple logistic regression models were performed for the dependent variable post-GDM/control status. Pearson correlation coefficient (r) was calculated for the correlation analysis of figure 3. To detect potential predictors for a low ISI a lasso linear regression model was used (16). Lasso regression is a penalized regression method and due to the shrinkage of the estimated regression coefficients it prevents an overfitting in the presence of multicollinearity of the predictors or high-dimensional data. In addition, lasso models perform a variable selection, because some of the regression coefficients shrunk exactly to zero. The dependent variable in the lasso model was the logarithmized ISI. The Schwarz Bayesian Information criterion was used as selection criterion to produce a sparse model and a 5-fold cross validation was used as a broader selection criterion. Linear regression models were applied for univariate and bivariate analyses of the relation between logarithmized ISI (dependent variable) and MRI/MRS variables. All statistical calculations were performed using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA) or R version 3.0.2 (http://www.R-project.org).

Results

Baseline characteristics

155 women 3 to 16 months after a pregnancy were consecutively enrolled during the study period. Eight women were excluded from further analysis due to a diagnosis of hyperthyroidism (n = 1), acute upper respiratory infection (n = 1), positivity for GAD65 and IA2 antibodies (n = 1), undocumented BMI (n = 3) or missing OGTT plasma glucose values (n = 2). The final sample consisted of 147 women, 96 women after GDM and 51 controls after a normoglycemic pregnancy. Baseline characteristics are shown in **Table 1**. Fifteen (15.6%) women after GDM had IFG, 13 (13.5%) IGT, 4 (4.2%) IFG+IGT and 2 (2.1%) T2D. Altogether 35.4% of women post GDM had maintained some kind of pathologic glucose tolerance (PGT) at study OGTT, their first after the pregnancy. Out of the control group, 3 women (5.9%) showed isolated IFG whereas the other participants were normoglycemic.

Metabolic differences between women with recent GDM and controls

In univariate logistic regression analyses for the dependent variable post-GDM/control status BMI ($P = .010$), waist circumference ($P = .007$), systolic ($P = .004$) and diastolic BP ($P = .046$), HDL cholesterol ($P = .04$) TSH ($P = .04$), ISI ($P < .001$), DI ($P < .001$), fetuin-A ($P < .001$) and leptin ($P = .001$) where significant predictors (**e-Table 3**). A lower DI (OR 0.995 (0.991–1.000); $P = .030$) and elevated plasma fetuin-A (OR 1.013 (1.002–1.024) $P = .023$) remained significantly associated with recent GDM in a multiple model that included all significant variables from the univariate analyses (**e-Table 4**). The ISI was not significant in the adjusted model but it has to be considered that it is used to calculate the DI and that several of the variables in the model (BMI, leptin, waist circumference, HDL cholesterol, systolic and diastolic BP) are also highly correlated with it, as shown two paragraphs below. When these variables were removed from the multiple model, a low ISI remained significantly associated with post-GDM status (OR 0.853 (0.762–0.954), $P = .0053$).

We repeated the regression analyses with only the normoglycemic study participants (baseline characteristics in **e-Table 5;** n = 110). In the multiple model, the DI remained significant (OR 0.995 (0.990–0.999); $P = .028$) whereas the p-value of fetuin-A increased to 0.08 (OR 1.010 (0.999–1.022)). Comparisons of the DI, insulin sensitivity and plasma fetuin-A in normoglycemic controls, normoglycemic women after GDM and women post GDM with PGT are also shown in **Figure 1**.

Factors associated with early pathologic glucose tolerance after GDM

We next examined how insulin sensitivity correlated to plasma glucose levels in women post GDM and in control subjects. First we estimated loess curves for the relation between the ISI and fasting (**Figure 2a**) and 2h OGTT plasma glucose (**Figure 2b**), separately for the post-GDM group and controls. We found that fasting and 2h glucose rose with lower insulin sensitivity in both cohorts. Fasting glucose in women post GDM at each level of insulin sensitivity was slightly higher than in controls. The 2h glucose was clearly higher in the post-GDM cohort, in particular at low levels of insulin sensitivity (**Figure 2b**). Using a segmented regression model we found a breakpoint at an ISI of 5.7 (95% CI: 3.6–7.9) in the ISI-2h plasma glucose curve for the women after GDM (**Figure 2c**). Below this breakpoint, the 2h plasma glucose rose sharply with further decreasing ISI values whereas above the breakpoint

Table 1. Baseline characteristics of women in the PPS-Diab study at the time of the study visit, 3–16 months after the index pregnancy, by study group.

group		post-GDM	controls	p-value
no. of subjects (*n*)		96	51	
clinical characteristics				
insulin during pregnancy		58 (60.4%)	-	
current glucose tolerance	NGT	62 (64.6%)	48 (94.1%)	**<0.001**$^{+}$
	IFG	15 (15.6%)	3 (5.9%)	
	IGT	13 (13.5%)	0	
	IFG + IGT	4 (4.2%)	0	
	T2DM	2 (2.1%)	0	
age (years) (mean ± SD)		35.9 ± 4.0	35.2 ± 3.9	0.531§
months post delivery (month) (mean ± SD)		9.1 ± 3.2	8.7 ± 2.3	0.483§
primiparous (n, %)		49 (51.0%)	28 (54.9%)	0.730$^{\#}$
breast feeding at time of visit (n, %)	full	6 (6.3%)	0	**0.009**$^{+}$
	partial	26 (27.1%)	25 (49.0%)	
	no	64 (66.7%)	26 (51.0%)	
smoking (n, %)	yes	8 (8.3%)	2 (3.9%)	0.495^{+}
	no	88 (91.7%)	49 (96.1%)	
	ex-smoker	31 (35.2%)	14 (29.2%)	0.568$^{\#}$
family history T2DM (first-degree relative) (n, %)	yes	27 (28.1%)	10 (19.6%)	0.257$^{\#}$
family history GDM (first-degree relative) (n, %)	yes	10 (10.4%)	2 (3.9%)	0.218^{+}
BMI (kg/m^2) (mean ± SD)		26.3 ± 6.3	23.6 ± 4.0	**0.009**§
waist circumference (cm) (mean ± SD)		83.5 ± 12.7 (*n* = 91)	77.7 ± 9.7	**0.007**§
systolic blood pressure (mmHg) (mean ± SD)		121.2 ± 10.8	115.4 ± 11.2	**0.004**§
diastolic blood pressure (mmHg) (mean ± SD)		76.1 ± 8.9	72.9 ± 9.1	0.051§
clinical chemistry				
triglycerides (mg/dl) (median (Q1--Q3))		71.0 (55.0–92.5)	62.0 (50.0–87.0)	0.155§
LDL cholesterol (mg/dl) (median (Q1--Q3))		103.5 (87.5–119.5)	108.0 (93.0–130.0)	0.088§
HDL cholesterol (mg/dl) (median (Q1--Q3))		61.0 (49.0–69.5)	62.0 (57.0–75.0)	**0.049**§
hsCRP		0.08 (0.02–0.31)	0.04 (0.01–0.11)	0.063§
ferritin (μg/liter) (median (Q1--Q3))		33.0 (19.0–47.0)	26.0 (16.0–45.0)	0.201§
γ-GT (U/liter) (median (Q1--Q3))		14.5 (12.0–20.5)	14.0 (11.0–18.0)	0.419§
TSH (μU/ml) (median (Q1--Q3))		1.7 (1.1–2.1)	1.8 (1.2–3.1)	0.092§
glucose metabolism				
fasting plasma glucose (mg/dl) (median (Q1--Q3))		93.5 (89.0–98.0)	90.0 (83.0–92.0)	**<0.001**§
plasma glucose 2 h (mg/dl) (median (Q1--Q3))		115.5 (99.5–132.5)	94.0 (82.0–110.0)	**<0.001**§
ISI (median (Q1--Q3))		4.2 (2.9–6.9)	6.6 (4.8–8.8)	**<0.001**§
ΔI 30′ (median (Q1--Q3))		42.9 (29.9–69.0)	42.3 (31.3–58.4)	0.527§
DI (median (Q1--Q3))		200.4 (151.9–280.8)	297.2 (215.9–368.3)	**<0.001**§
protein mediators				
fetuin A (median (Q1--Q3))		294.3 (271.5–330.1)	263.6 (242.6–295.0)	**<0.001**§
leptin (median (Q1--Q3))		10.8 (6.9–16.3)	6.7 (2.8–11.6)	**<0.001**§
adiponectin (median (Q1--Q3))		9.4 (6.9–14.4)	11.7 (9.3–14.7)	0.120§
resistin (median (Q1--Q3))		8.4 (6.8–10.7)	9.0 (7.6–11.0)	0.464§
NEFA (median(Q1-Q3))		587.0 (461.5–685.5)	555.0 (444.0–711.0)	0.517§

$^{+}$ Fisher Exact test; $^{\#}$ χ-square test; § Mann-Whitney-*U* test.

only a weak linear relationship was identified. All women with T2D and IGT, and most women with IFG had an ISI below the breakpoint. We could not identify a breakpoint in the control group. To identify other factors associated with PGT early after GDM we undertook univariate logistic regression analyses for PGT vs. NGT in the post-GDM cohort. In addition to the ISI BMI, waist circumference, triglycerides, HDL cholesterol, fetuin-A, leptin,

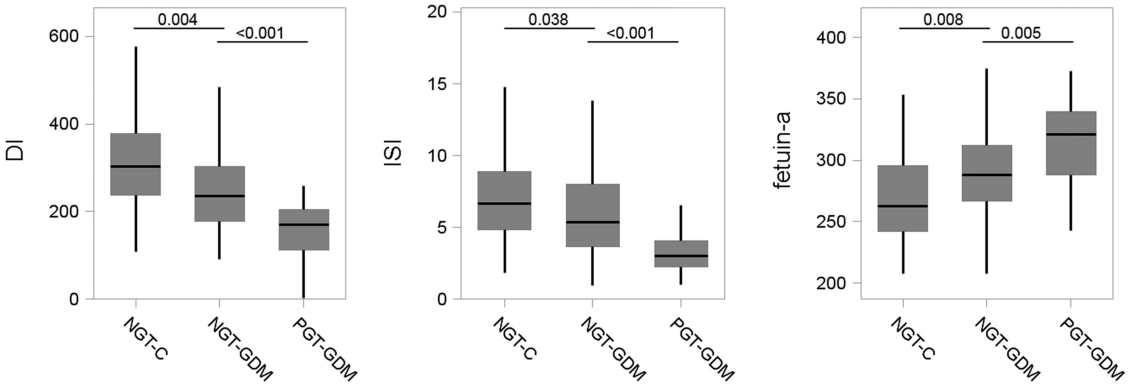

Figure 1. DI, ISI and fetuin-A in normoglycemic controls (NGT-C), normoglycemic women post GDM (NGT-GDM) and women post GDM with pathologic glucose tolerance (PGT-GDM). Kruskal-Wallis test over all groups with $P < .0001$ for all 3 variables; p-values of subsequent pairwise comparisons (Mann Whitney-U test) between NGT-C and NGT-GDM, as well as between NGT-GDM and PGT-GDM shown in the diagrams

gamma glutamyl transferase, nonesterified fatty acids (NEFA) and the DI were significant in this analysis (**e-Table 6**). In summary, we found that a low ISI, other components of the metabolic syndrome and elevated fetuin-A levels were associated with pathologic glucose tolerance in women post GDM.

Factors predictive of low insulin sensitivity

Because we had seen that low insulin sensitivity was associated with a history of GDM and, among the women post GDM, with early PGT, we next tested which factors were most predictive of a low ISI in our cohorts. We used a lasso regression model to identify variables predictive of a low $\log_{ISI}$ (log transformation because of the non-normally distributed variable ISI) and performed this analysis for the whole study population, only for participants normoglycemic after pregnancy and separately for the post-GDM cohort, respectively. We used the Schwarz Bayesian Information Criterion and 5-fold Cross Validation for variable selection. The results of these analyses are summarized in **Table 2. E-figure 4** shows a visual description of the lasso model selection iterations. Four variables were strong independent predictors of a low ISI that were consistent over all three tests: High plasma leptin, BMI, waist circumference and plasma triglycerides. Additionally, in the two analyses that included both women post GDM and controls, post-GDM status was independently predictive of low insulin sensitivity. Of all variables tested, plasma leptin had the strongest BMI-independent association with the ISI (BMI-adjusted r= −0.36; $P < .0001$; **Figure 3**).

Differences in body composition in women post GDM and controls

To test potential associations of body composition and ectopic lipids with a recent history of GDM a representative subcohort of study participants also underwent MRI/MRS examinations. We measured body fat distribution and liver and muscle lipid content in 42 women post GDM and 24 controls (**baseline characteristics in e-Table 7**). In nonparametric testing and univariate regression analyses, the only MRI/MRS parameter significantly different between the groups and was a higher liver fat content in the women post GDM (**e-Table 7** and data not shown). However, in a multiple logistic regression model together with BMI high liver fat was no longer significantly predictive of the post-GDM status ($P = .2$). We saw no significant difference between the two groups in any of the MRI/MRS parameters when we examined only the normoglycemic study participants.

Body composition and low insulin sensitivity

In univariate linear models, in all post-GDM and control subjects combined, only in the normoglycemic study participants as well as only in the post-GDM group, intraabdominal fat volume and liver fat content were predictive of $\log_{ISI}$. However, in multiple linear regression models adjusted for BMI none of the parameters remained

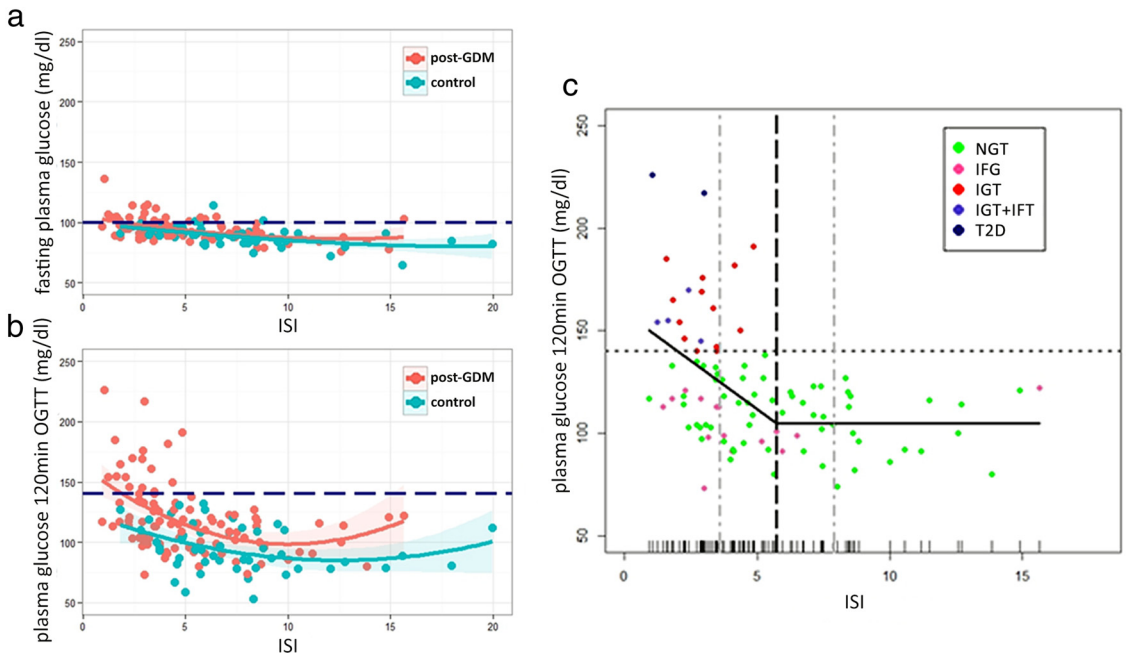

Figure 2. (**a, b)** Relationship between ISI and fasting plasma glucose (a) and ISI and plasma glucose at 120 minutes of the OGTT (b) in women post GDM (in red) and in control subjects (in blue). The solid lines are the Loess curves and the shaded bands around the each Loess curve indicate the 95% confidence interval (CI). The dashed lines indicate the upper limits of normal glucose values. (**c**) Segmented linear regression fit to the plasma glucose 120 minutes OGTT value and ISI in the post-GDM group.

Table 2. regression analysis with LASSO selection; dependent variable low log_{ISI}; 3 different samples of the study population. All variables selected by 5-fold Cross Validation are shown. The variables additionally selected by the Schwarz Bayesian Information Criterion are printed in italics. Variables consistent across the 3 analyses are printed in boldface. (-) indicates inverse associations.

all post-GDM and controls	normoglycemic post-GDM and controls	post-GDM only
n = 147	*n* = 110	*n* = 96
Leptin	***leptin***	***leptin***
BMI	***BMI***	***BMI***
Triglycerides	**triglycerides**	***waist circumference***
waist circumference	**waist circumference**	***triglycerides***
post-GDM vs. control status	post-GDM *vs.* control status	*gamm gt.*
γ gt.	age	*hdl cholesterol (-)*
NEFA	smoking status	NEFA
hdl cholesterol (-)	adiponectin (-)	fetuin a
adiponectin (-)		family history GDM
diastolic bp		breastfeeding status
systolic bp		diastolic bp

significant or improved the overall predictive capacity of the model in any of the 3 samples studied (**Table 3** and data not shown). Additionally, when we again used lasso selection with the MRI parameters and BMI, leptin, triglycerides and post-GDM/control status as the input variables, none of the MRI variables was chosen by the model as a relevant predictor of insulin sensitivity. BMI, leptin and triglycerides however were again selected consistently (data not shown).

In summary, liver fat content and abdominal visceral adiposity were predictive of low insulin sensitivity but these associations were lost after adjustment for BMI or other variables that we had previously found to be related to insulin sensitivity in this study.

Discussion

Our main findings were that, compared to control subjects, women who had GDM during a recent pregnancy had a reduced DI, higher levels of plasma fetuin-A and lower insulin sensitivity. Low insulin sensitivity was also the major determinant of pathologic glucose tolerance in the women after GDM. The factors consistently and independently predictive of low insulin sensitivity in our study were high plasma leptin, BMI, plasma triglycerides and waist circumference. Ectopic fat in the liver was predictive of post-GDM status only in univariate analyses but this association was lost after adjustment for BMI. Similarly, ectopic fat in the liver and abdominal visceral adipose tissue were predictive of low insulin sensitivity only in univariate analyses but not after adjustment for BMI or other relevant variables.

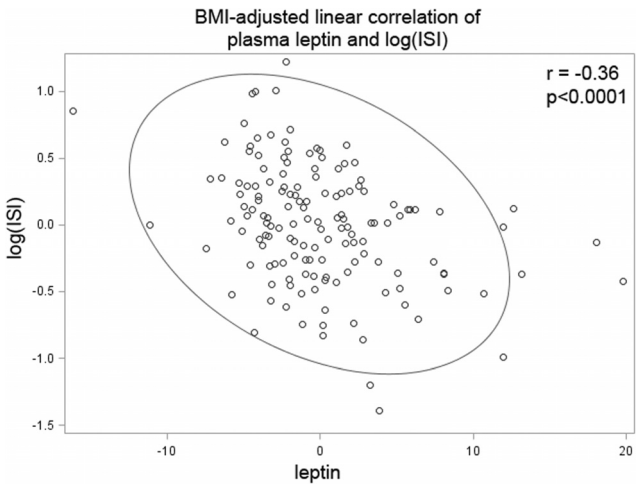

Figure 3. linear correlation analysis of fasting plasma leptin level and log_{ISI}, adjusted for BMI (analysis of all study participants)

The first aim of this study was to describe the metabolic phenotype of a specific population of young individuals at high risk for T2D, namely women with recent GDM. GDM was used as selection criterion because human pregnancy represents a stress test of glucose metabolism and identifies individuals with a predisposition for T2D. Women after GDM have an about 10-fold increased risk for T2D compared to women after a normoglycemic pregnancy (18.9 vs. 2.0% within 9 years; 10).

One major difference between women post GDM and controls in our study was that the group after GDM had a lower DI, even the women who were again normoglycemic after pregnancy. This finding demonstrates that impaired beta cell function is already present in young at-risk individuals and does not only develop after prolonged insulin resistance, as suggested for example by the Whitehall II study (3, 17). What causes this early beta cell defect is unclear. It was not linked to a family history of T2D in our study but nevertheless could have genetic, as well as epigenetic and environmental reasons.

In addition to a lower DI, a higher plasma level of the hepatokine fetuin-A was strongly associated with post-GDM status. This association missed multivariate significance when only the normoglycemic study participants were examined ($P = .08$). However we believe this was

Table 3. parameters from the MRI/MRS substudy; p-values and adjusted R2 values for crude and BMI-adjusted linear regression models with low log_{ISI} as dependent variable; analysis of all study participants

parameter	crude model		adjusted for BMI	
	p-value	adjusted R^2	p-value	adjusted R^2
BMI	**<0.001**	0.458	-	-
intraabdominal fat	**<0.001**	0.331	0.455	0.454
liver fat	**0.003**	0.189	0.881	0.450
intramyocellular lipids in soleus muscle	0.457	0.075	0.471	0.454
intramyocellular lipids in tibialis anterior muscle	0.967	0.067	0.427	0.455
ratio intraabdominal fat / Subcutaneous fat	0.670	0.069	0.514	0.453
ratio intraabdominal fat / total body fat	0.063	0.117	0.825	0.450

mainly the result of the reduced sample size because the multivariate OR remained almost unchanged and univariate significance remained high ($P = .008$). An association of elevated fetuin-A levels with GDM has been found previously in one study (18) but not in another (19). Fetuin-A also has been shown to be a biomarker predictive of future T2D. It is involved in inflammatory signaling via TLR4 and therefore might be relevant for the pathogenesis of both GDM and T2D (20).

Insulin sensitivity was slightly lower in normoglycemic women post GDM than in controls and substantially reduced in those women who maintained PGT after GDM. Overall, central metabolic characteristics show stepwise differences from women normoglycemic during pregnancy over women normoglycemic after GDM to women with a persistent impairment of glucose metabolism after GDM (**Figure 1**).

Looking at the predictors of low insulin sensitivity in our study, an unexpected finding was that high plasma leptin was associated with low insulin sensitivity in a BMI-independent manor. In rodents leptin has mainly been seen as an insulin sensitizing hormone. In humans however, conclusive evidence on the relationship between leptin and insulin sensitivity in subjects without congenital leptin deficiency or severe hyperleptinemia is lacking (21). Higher leptin levels in insulin resistant subjects could be the result of adipose tissue IR but also of more leptin resistance of the brain. In accordance with the concept of a brain-centered glucoregulatory system (22) central leptin resistance could in fact cause insulin resistance. Some data also suggest a direct induction of muscle insulin resistance by leptin in humans (23). Thus a primary, e. g. genetic, predisposition for higher leptin levels might also be directly causative for low insulin sensitivity.

Elevated triglycerides in insulin resistant subjects, as we see it in our study, are generally interpreted as a consequence of the insulin resistant liver overproducing very low density lipoprotein (VLDL) particles (24, 25). However, recent data suggest that this alteration may also contribute to the induction of IR (26).

Having had GDM was also an independent predictor of a low ISI. This suggests a predisposition for low insulin sensitivity in the post-GDM cohort beyond the parameters we measured. Like the lower DI, this might be determined genetically or by epigenetic or environmental factors.

Ectopic fat has long been considered a major risk factor for T2D and a driver of IR (3). We found no BMI-independent associations of ectopic lipids with post-GDM status or low insulin sensitivity in the MRI substudy of this project, which included a representative sample of 45% of all study participants. On the other hand, waist circumference, a clinical surrogate for abdominal visceral adiposity, was a weak but independent predictor of low insulin sensitivity in the whole study cohort. Previous studies showed associations between liver and muscle triglycerides measured by MRS and IR in women after GDM (27, 28, 29). These projects lacked the consecutive parallel recruitment of women post GDM and controls that is a strength of our current study but we cannot conclude that this explains the different results. The causative role of steatosis of the liver in hepatic IR and intramyocellular triglycerides in muscle IR has recently also been questioned by others (6, 30). Additionally, rather than the overall quantity of organ triglycerides it might be particularly reactive lipid species in distinct cellular compartments that actually contribute to pathologic IR (4, 31). Taken together our results do not exclude a contribution of ectopic lipids to diabetes pathogenesis in the at-risk individuals under investigation. But they suggest that such a contribution would be relatively small and that other factors are more relevant.

The second aim of this study was to identify factors associated with the persistence of pathologic glucose tolerance after GDM. In this respect we found a low insulin sensitivity to be most relevant. It was associated with several other components of the metabolic syndrome. This is in line with previous work from another group (32) and has implications for T2D prediction and preventive measures in women after GDM.

Strengths of our study are the consecutively recruited uniform cohorts with little confounding medication or concomitant diseases and the detailed clinical phenotyping that was done.

The main limitation of our study is its cross sectional

observational design that cannot prove causality. Further human studies are necessary to clarify the cause-effect relationships in the associations reported here. Another limitation of our approach is that metabolic changes as a consequence of the preceding GDM cannot be excluded. With respect to the MRI substudy, a selection bias cannot be fully ruled out despite consecutive recruitment and similar baseline characteristics compared to the whole study cohort. Finally, it is unclear to what extent findings from a cohort of young women can be transferred to the general population.

In summary, we describe important components of the diabetes risk phenotype of women with recent GDM and factors associated with early pathologic glucose tolerance after GDM. Our data support the hypothesis that inadequate beta cell function and alterations in fetuin-A and leptin signaling contribute to the pathogenesis of T2D. At this disease stage these factors may be equally or even more important than ectopic lipids and low-grade inflammation. Early T2D therefore might be more a disorder of hormonal dysregulation than a disease resulting from toxic metabolites. This hypothesis warrants testing in further human studies because it suggests novel approaches to T2D risk prediction and prevention.

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