*Journal of Developmental Origins of Health and Disease*

**www.cambridge.org/doh**

# A combined *in vivo* and *in silico* model shows specific predictors of individual trans-generational diabetic programming

Claudia Eberle[1,2] and Christoph Ament[3]

[1]Hochschule Fulda – University of Applied Sciences, Medicine with Specialization in Internal Medicine and General Medicine, 36037 Fulda, Germany; [2]Diabetes Center and Department of Internal Medicine IV of the Ludwig-Maximilians University of Munich (LMU), 80336 München, Germany and [3]Chair of Control Engineering, University Augsburg, 86159 Augsburg, Germany

## Original Article

**Cite this article:** Eberle C and Ament C. (2021) A combined *in vivo* and *in silico* model shows specific predictors of individual trans-generational diabetic programming. *Journal of Developmental Origins of Health and Disease* **12**: 396–403. doi: 10.1017/S2040174420000471

Received: 19 April 2018
Revised: 26 March 2020
Accepted: 13 May 2020
First published online: 18 August 2020

**Keywords:**
gestational diabetes; hyperglycemia; trans-generational programming; Ins2Akita mouse model; in silico model; model-based predictors

**Address for correspondence:**
Claudia Eberle, Hochschule Fulda – University of Applied Science, Medicine with specialization in Internal Medicine and General Medicine, Leipziger Str. 123, 36037 Fulda, Germany.
Email: claudia.eberle@hs-fulda.de

## Abstract

Diabetic pregnancies are cleary associated with maternal type 2 diabetes and metabolic syndrome as well as atherosclerotic diseases in the offspring. The global prevalence of hyperglycemia in pregnancy was estimated as 15.8% of live births to women in 2019, with an upward trend. Numerous parental risk factors as well as trans-generational mechanisms targeting the utero-placental system, leading to diabetes, dysmetabolic and atherosclerotic conditions in the next generation, seem to be involved within this pathophysiological context. To focus on the predictable impact of trans-generational diabetic programming, we studied age- and gender-matched offspring of diabetic and nondiabetic mothers. The offspring generation consists of three groups: C57BL/6-J-*Ins2*$^{Akita}$ (positive control group), wild-type C57BL/6-J-*Ins2*$^{Akita}$ (experimental group), and C57BL/6-J mice (negative control group). We undertook intraperitoneal glucose tolerance tests at 3 and 11 weeks of age. Moreover, this *in vivo* model was complemented by a corresponding *in silico* model. Although at 3 weeks of age, no significant effects could be observed, we could demonstrate at 11 weeks of age characteristic and significant differences in relation to maternal diabetic imprinting based on the *in silico* model-based predictors. These predictors allow the generation of a concise classification tree assigning maternal diabetic imprinting correctly in 91% of study cases. Our data show that hyperglycemic *in utero* milieu contributes to trans-generational diabetic programming leading to impaired glucose tolerance in the offspring of diabetic mothers early on. These observations can be clearly and early distinguished from genetically determined diabetes, for example, type 1 diabetes, in which basal glucose values are significantly raised.

## Introduction

Diabetic pregnancies are associated with maternal type 2 diabetes (T2D) after delivery as well as T2D, metabolic syndrome, and atherosclerotic diseases in their offspring early in life.[1–3] The global prevalence of hyperglycemia in pregnancy (HIP) was 15.8% of live births to women in 2019 referring to the International Diabetes Federation (IDF)[2] – with an upward trend and an increased estimated number of unreported cases. HIP comprises pregestational diabetes in pregnancy, which may play a role in approximately 7.9% of HIP cases, other types of diabetes first detected in pregnancy in approximately 8.5% of HIP cases as well as gestational diabetes mellitus (GDM), which affect approximately 83.6% of HIP cases.[2] The HIP prevalence varies in different IDF world regions between 7.5% and 27.0%,[2] depending on risk factors like maternal age, body mass index, diabetic history as well as ethnic, social, and income groups.[1,3] Many authors postulate that maternal diabetes accompanied by obesity seems to be the main driving metabolic programming issue in terms of dysmetabolic conditions in their offspring. In contrast, our data will clearly indicate that maternal diabetes per se (without accompanied obesity) seems to be a strong risk or imprinting factor in terms of diabetic programming leading to diabetes as well as metabolic and cardiovascular diseases in the offspring early in life.

Since it is not explicitly known how maternal diabetic *in utero* environment imprints the fetus for diabetes and metabolic syndrome as well as atherosclerotic disease later in life, we analyzed the *Ins2*$^{Akita}$ mouse model, in which the offspring of diabetic mothers (DMs) develop diabetes, accompanied by altered insulin sensitivities as well as abnormal glucose-stimulated insulin release.

Focusing on epigenetic or other aspects, multiple mechanisms of *in utero* and perinatal programming may play a key role. "Perinatal programming" defines the perturbation at one or more critical periods during development causing persistent alterations with sometimes irreversible consequences.[3] To characterize and assess mechanisms of diabetic programming, appropriate predictors as well as therapeutic approaches have to be found.



CAMBRIDGE UNIVERSITY PRESS

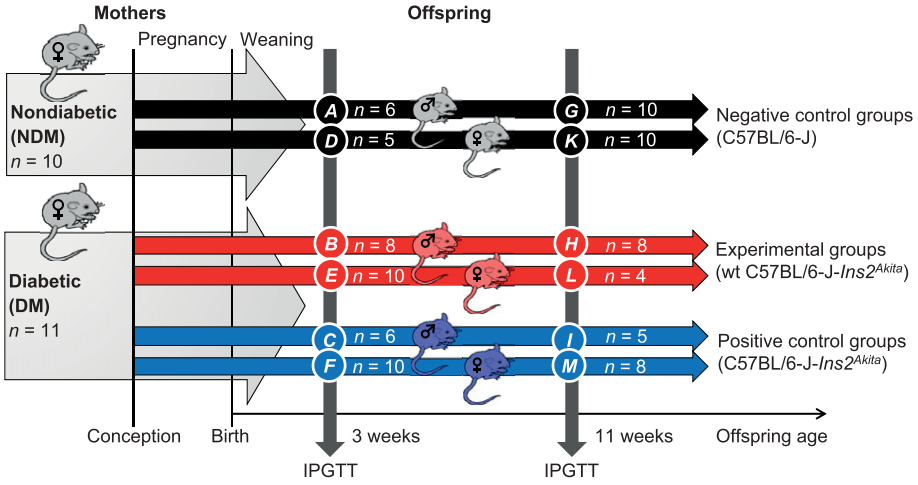



**Fig. 1.** Setup of the *in vivo* experiment. Offspring were assigned by polymerase chain reaction to negative control (black), experimental (red), and positive control (blue) groups; in addition, offspring were classified and matched by age as well as sex into groups (*A*) to (*M*), and the number of subjects in each group is denoted with *n*.

We have recently proposed a newly established dynamic *in silico* model and have shown that parameter identification provides individual predictors characterizing different types of metabolic diseases already at an early stage.[4,5] We showed that model parameters such as short-term and long-term glucose sensitivities, $k_{G1}$ and $k_{G2}$, respectively, are suitable for significantly predicting metabolic developments at early stages for subjects within one generation.

In this contribution, we will extend this approach by targeting trans-generational diabetic *in utero* programming. We have to identify specific individual predictors, such as $k_{G1}$ and $k_{G2}$, for diabetic development in offspring and analyze their relationship to maternal diabetic imprinting.

We will show that *in silico* model-based predictors are an effective approach to obtain significant results even in the case of small-group sizes, including high sensitivity as well as high specificity.

## Method

### In vivo *model*

The $Ins2^{Akita}$ mouse model presents an established diabetic model with appealing characteristics, such as altered insulin sensitivity as well as abnormal glucose-stimulated insulin release.

Due to a misfolded insulin-2 protein, which causes endoplasmic reticulum stress and β-cell death, insulin processing is disrupted, which results in the early development of hyperglycemia. Additionally, by using $Ins2^{Akita}$ mice, the mechanisms of type 2 diabetes could be investigated without the associated interference of obesity.

Within the experimental setup shown in Fig. 1, a group of DMs is established based on the C57BL/6-J-$Ins2^{Akita}$ mouse model. They are compared to matched C57BL/6-J mice, which are denoted as nondiabetic mothers (NDMs). Both groups have equivalent conditions prior to and during pregnancy. Body weight and plasma glucose levels are controlled on a daily basis during pregnancy. There are no relevant differences in body weight, neither pre-conceptional nor during the first 13 days of pregnancy. However, in the final stage of pregnancy, the gestational weight gain of NDM is higher than that of DM (NDM 39.0 ± 3.0 g vs. DM 31.6 ± 2.4 g at day 19 of pregnancy, denoting mean ± standard deviation). As expected, the fasting plasma glucose levels in DM are raised compared to NDM during the whole period of pregnancy (NDM 151.6 ± 17.5 mg/dl vs. DM 179.7 ± 23.9 mg/dl, denoting mean ± standard deviation).

This study focuses on specific trans-generational diabetic programming aspects, which are defined as not directly genetically determined impacts from mothers to their offspring. Hence, the offspring of DMs who were not genetically determined to diabetes (wild type; wt C57BL/6-J-$Ins2^{Akita}$) are classified as the *experimental group*. They are compared to the offspring of NDMs who are neither genetically determined nor affected by diabetic programming (C57BL/6-J as a *negative control group*). To allow a comparison to genetically moderated mechanisms, a *positive control group* of genetically determined offspring (C57BL/6-J-$Ins2^{Akita}$) of DM is also considered. Fig. 1 gives a survey of the three resulting offspring groups.

All offspring were raised under equivalent experimental conditions. Their body weights and fasting glucose concentrations were measured regularly. At 3 and 11 weeks of age, an intraperitoneal glucose tolerance test (IPGTT) was performed for all offspring. The experimental setup and the definition of the groups (*A*) to (*M*) for female and male offspring at 3 and 11 weeks of age, including their sizes *n*, are summarized in Fig. 1.

### In silico *model*

*In silico* models can be used to simulate and characterize dynamical processes, for example, glucose insulin dynamics during an IPGTT. A survey of appropriate models in diabetes is given by Cobelli *et al.*[6] To provide an appropriate *in silico* model, we redefined Bergman's "minimal model"[7,8] as developed in Eberle *et al.*[4] The block diagram of the resulting nonlinear model is displayed in Fig. 2 within the green panel using transfer functions (shown as white blocks). It comprises the functional compartments of the plasma, interstitial tissue, pancreatic controller, and external inputs. All components can be assigned to either the glucose subsystem (upper part) or the insulin subsystem (lower part). Two external inputs, an intraperitoneal glucose bolus $u_{GP}$ and an intraperitoneal insulin bolus $u_{IP}$, are provided. To simulate an IPGTT, an impulse function at $t = 0$ min is applied to $u_{GP}$, whereas $u_{IP}$ remains zero. The simulated plasma glucose concentration $\hat{G}$ serves as model output (Fig. 2).

### *Identification of* in silico *model-based predictors*

In the approach presented here, the measured IPGTT data retrieved from the *in vivo* model are used for the identification of individual *in silico* models. For each subject, the measured plasma glucose values $G$ are compared with the simulated glucose

https://doi.org/10.1017/S2040174420000471 Published online by Cambridge University Press

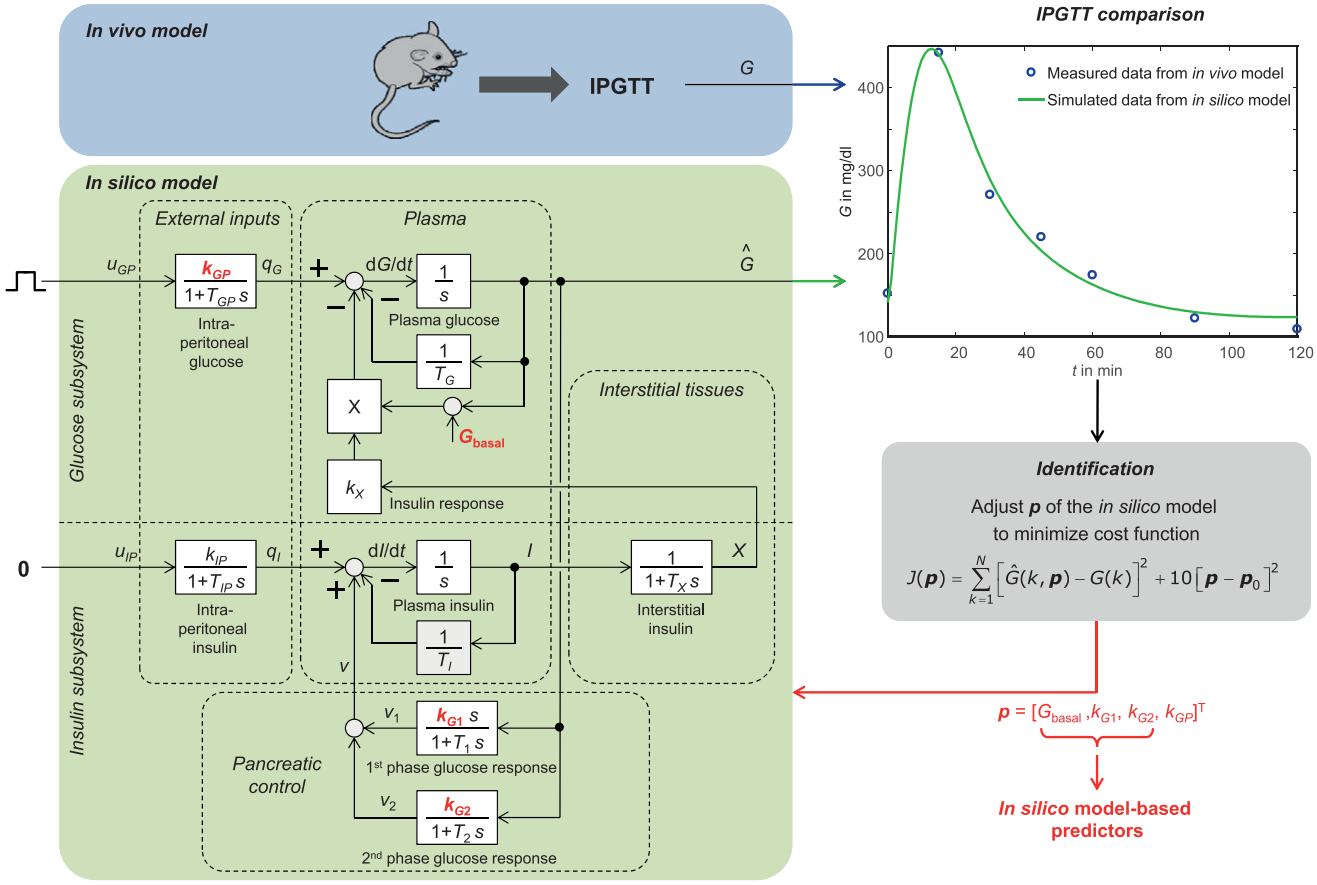



**Fig. 2.** Block diagram of the *in silico* model comprising functional compartments of the plasma, interstitial tissue, pancreatic control, intraperitoneal tissue, and external inputs (in green). Simulated glucose values $\hat{G}$ are compared to measured values $G$ from the *in vivo* model (in blue). For identification, a cost function $J$ is minimized (gray box) with respect to the *in silico* model parameters $p$ (in red). Finally, they serve as predictors.

values $\hat{G}$ (see Fig. 2). A cost function $J$ is defined to assess their deviations:

$$J(\boldsymbol{p}) := \sum_{k=1}^{N} \left[\hat{G}(k,\boldsymbol{p}) - G(k)\right]^2 + 10\,[\boldsymbol{p} - \boldsymbol{p}_0]^2.$$

In the first term, the squared differences between $G$ and $\hat{G}$ are summed over the $N = 7$ measurements during an IPGTT at time steps $t = 0$, 15, 30, 45, 60, 90, and 120 min. The second term considers the squared difference of the current parameter vector $\boldsymbol{p}$ to its initial definition $\boldsymbol{p}_0$. In $\boldsymbol{p}$, the four most relevant *in silico* model parameters

$$\boldsymbol{p} = [G_{\text{basal}},\ k_{G1},\ k_{G2},\ k_{GP}]^T$$

are selected. They are printed in red in the *in silico* model block diagram of Fig. 2. We proved the identifiability of this set of parameters $\boldsymbol{p}$ according to.[9,10] For diagnostic purposes, we will use the first three parameters:

- the basal plasma glucose $G_{\text{basal}}$;
- the first-phase (short term) glucose response gain $k_{G1}$; and
- the second-phase (long term) glucose response gain $k_{G2}$.

The fourth parameter, the gain of the intraperitoneal glucose dosage $k_{GP}$, only serves as an auxiliary parameter for model adjustment, which will improve identification accordance.

For each subject, a simplex optimization algorithm[11] implemented in MATLAB© is applied to solve the optimization problem by minimizing the cost function $J$ and to fit the *in silico* model to the subject's measurement in the best possible way.

Finally, an optimized $\boldsymbol{p}$ is available for each subject, providing *in silico* model-based predictors (Fig. 2) for the two subsequent analysis procedures.

## Pooled predictors and their effects

How specific are the *in silico* model-based predictors retrieved only from offspring when they are applied to forecast maternal imprinting? Statistical measures can be applied. To assess the impact of a feature difference between groups, we suggest its *effect* $e$, which is defined as the mean $\overline{y}_2$ of a subset of subjects holding this feature compared with the mean $\overline{y}_1$ of a subset comprising subjects without the feature:

$$e = \overline{y}_2 - \overline{y}_1.$$

This measure is commonly used in the design of experiments field.[12] For example, to calculate the effect of the offspring sex at 11 weeks of age, $\overline{y}_2$ is calculated from the mean of all female groups (*K*), (*L*), (*M*) with $n = 22$, and $\overline{y}_1$ is calculated from all male groups (*G*), (*H*), and (*I*) with $n = 23$. In this way, larger group sizes $n$ are achieved, and a double-sided $t$-test can be applied to determine the level of significance $P_e$ of the corresponding effect $e$.

https://doi.org/10.1017/S2040174420000471 Published online by Cambridge University Press

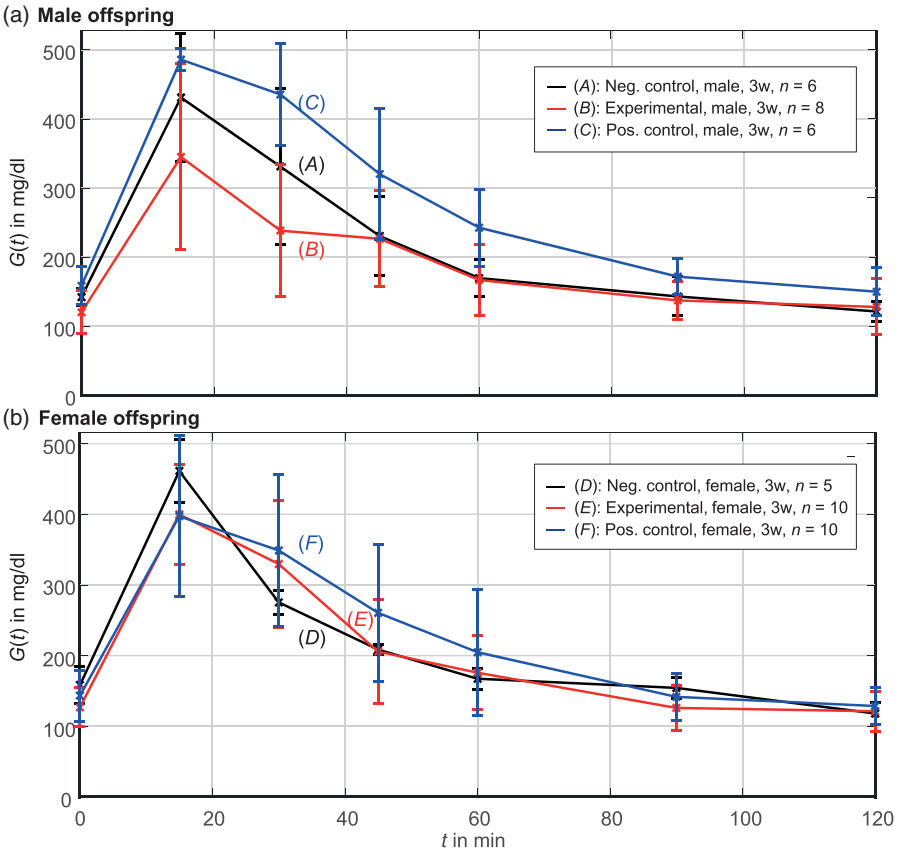



**Fig. 3.** Mean and standard deviation (as error bars) of plasma glucose $G(t)$ during IPGTT for all groups $(A)$ – $(F)$ at 3 weeks of age (male and female offspring).

### *Classification of individual predictors*

A classification of individual predictors may serve as an alternative method that does not require static methods and, therefore, is independent of certain group sizes that are necessary for significance. We use, for example, the $n = 45$ data sets at 11 weeks of age (all subjects included in groups $(G)$ to $(M)$) to train a *classification tree*.[13] Each data set comprises the predictors $\{G_{\text{basal}}, k_{G1}, k_{G2}\}$ and the known maternal class. The training was performed with the Statistics and Machine Learning Toolbox of MATLAB©.

Thereafter, classification success can be presented as *confusion matrix* $C$, which counts all $N$ data sets referring to the known classes assigned as rows and to the predicted classes assigned as columns. Correct classifications are counted as main diagonal elements, and their share of all data sets is

$$P_c = \text{trace}(C)/n.$$

## Results

### In vivo *model*

The plasma glucose development $G(t)$ during IPGTT shows a positive deflection as a response to the intraperitoneal glucose administration at $t = 0$ min in all cases. At 3 weeks of age (Fig. 3), a high maximum amplitude of approximately 400 mg/dl at $t = 15$ min and a complete remission to basal values at $t = 120$ min are observed for all offspring groups. The levels of the female groups are almost equal, whereas the male groups $(A)$, $(B)$, and $(C)$ tend to differ at $t = 30$ min.

At 11 weeks of age (Fig. 4), the observation is different: both negative control groups $(G)$ and $(K)$ of NDMs show an improved and physiological glucose tolerance with a peak value below 300 mg/dl and a complete remission after 120 min. The positive control groups $(I)$ and $(M)$, however, show a diabetic profile, with increased basal glucose levels and an incomplete remission. The experimental groups $(H)$ and $(L)$, in turn, present a characteristic that is different from that of both control groups: within the short-term reaction (15–45 min), glucose levels are increased similarly to the positive control groups, while on the long-term time scale (60–120 min), it decreases to the physiological values of the negative control groups.

### In silico *model-based predictors*

The application of the *in silico* model allows condensing these qualitative observations into quantitative predictors. In Table 1, selected combined groups are considered, and the mean and standard deviation for the model-based predictors $\{G_{\text{basal}}, k_{G1}, k_{G2}\}$ are shown.

The best values are obtained in the negative control group at 11 weeks of age (line 1). The mean basal glucose value $G_{\text{basal}}$ is low, and the glucose sensitivities $k_{G1}$ and $k_{G2}$ are high. This case may serve as a reference and is most similar to the human reference values (line 5). The experimental group (line 2) shows an elevated $G_{\text{basal}}$ and a slightly reduced $k_{G2}$, which is still sufficient for remission in the IPGTT. However, it is characterized by a small $k_{G1}$ that causes a distinct peak in IPGTT. The positive control group (line 3) shows a high value of $G_{\text{basal}}$ combined with minimum values of $k_{G1}$ and $k_{G2}$, which describes a diabetic profile.

During weaning at 3 weeks of age, $G_{\text{basal}}$ is slightly elevated and $k_{G1}$ is reduced (line 4 compared to line 1). However, the

https://doi.org/10.1017/S2040174420000471 Published online by Cambridge University Press

**Table 1.** Different IPGTT patterns are observed during weaning (offspring 3 weeks of age) as well as later (offspring 11 weeks of age) for negative control, positive control, and experimental groups. Means $\mu$ and standard deviations $\sigma$ of combined groups are shown. The negative control group at 11 weeks of age may serve as a reference (gray background)

| # | Combined groups | $G_{basal}$ (mg/dl) | | $k_{G1}$ (µU/ml · dl/mg) | | $k_{G2}$ (µU/ml · dl/mg/min) | |
|---|---|---|---|---|---|---|---|
| | | $\mu$ | $\sigma$ | $\mu$ | $\sigma$ | $\mu$ | $\sigma$ |
| 1. | Negative control (11 weeks) (*G*) + (*K*), $n = 20$ | 115.757 | ±21.066 | 13.377 | ±9.725 | 0.119 | ±0.056 |
| 2. | Experimental control (11 weeks) (*H*) + (L), $n = 12$ | 136.051 | ±27.109 | 1.949 | ±1.024 | 0.081 | ±0.029 |
| 3. | Positive control (11 weeks) (*I*) + (*M*), $n = 13$ | 318.246 | ±84.341 | 0.901 | ±1.043 | 0.044 | ±0.031 |
| 4. | Weaning (3 weeks) (*A*) – (*F*), $n = 45$ | 139.879 | ±31.247 | 5.051 | ±6.931 | 0.142 | ±0.087 |
| 5. | Human reference values[10] | 90.000 | | 20.000 | | 0.110 | |

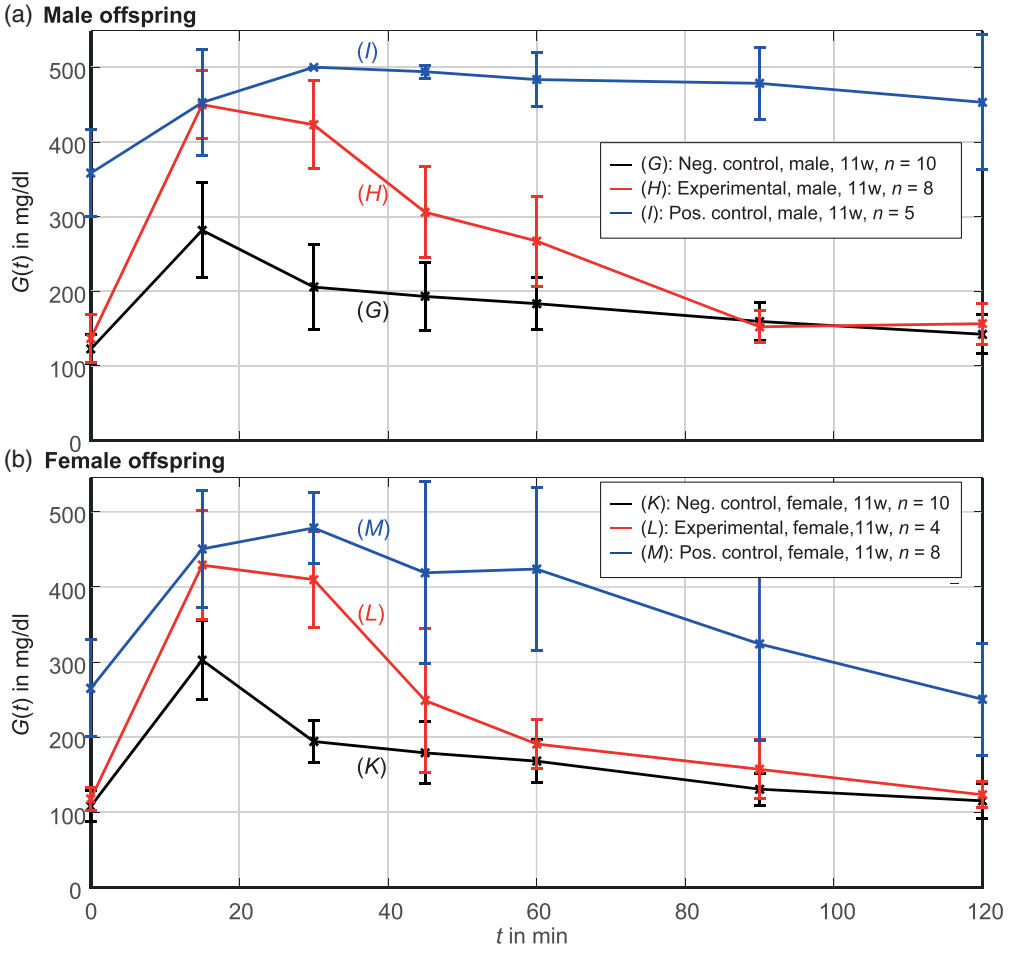



**Fig. 4.** Mean and standard deviation (as error bars) of plasma glucose $G(t)$ during IPGTT for all groups (*G*) – (*M*) at 11 weeks of age (male and female offspring).

long-term glucose sensitivity $k_{G2}$ performs better, which leads to a stable remission in IPGTT.

### *Pooled predictors and their effects*

To assess these observations, the effects and significances of pooled predictors are summarized in Table 2 for different impacts. During weaning at 3 weeks of age, the effects between the experimental and both control groups are small and (with one exception) not significant (lines 1–3). In contrast, all effects are significant at 11 weeks of age (lines 4–6). In the experimental group, $G_{basal}$ is elevated compared to the negative control group (line 4) but is much better than that in the positive control group (line 5). Gains $k_{G1}$ and $k_{G2}$ are severely degraded in the experimental group compared to the negative control group (line 4) but are still better than that in the positive control group (line 5). Line 6 just proves significant differences between both control groups; its effects are equal to line 4 minus line 5. Finally, the impact of offspring sex is analyzed in lines 7 and 8.

https://doi.org/10.1017/S2040174420000471 Published online by Cambridge University Press

**Table 2.** Effects $e$ of the predictors $\{G_{basal}, k_{G1}, k_{G2}\}$ and their levels of significance $P_e$ are shown. Significant predictors with a level of $P_e \leq 0.05$ are printed in bold.

| # Group relations | $G_{basal}$ (mg/dl) | | $k_{G1}$ (μU/ml · dl/mg) | | $k_{G2}$ (μU/ml · dl/mg/min) | |
|---|---|---|---|---|---|---|
| | $e$ | $P_e$ | $e$ | $P_e$ | $e$ | $P_e$ |
| Maternal impact on offspring at 3 weeks of age | | | | | | |
| 1. Experimental to negative control groups (3 weeks) (*B*, *E*) to (*A*, *D*), $n$ = 18 to 11 | −18.814 | 0.081 | −0.499 | 0.725 | 0.023 | 0.455 |
| 2. Experimental to positive control groups (3 weeks) (*B*, *E*) to (*C*, *F*), $n$ = 18 to 16 | **−26.883** | **0.021** | −0.548 | 0.844 | 0.024 | 0.486 |
| 3. Positive to negative control groups (3 weeks) (*C*, *F*) to (*A*, *D*), $n$ = 16 to 11 | 8.069 | 0.469 | 0.049 | 0.988 | −0.001 | 0.978 |
| Maternal impact on offspring at 11 weeks of age | | | | | | |
| 4. Experimental to negative control groups (11 weeks) (*H*, *L*) to (*G*, *K*), $n$ = 12 to 20 | **20.294** | **0.024** | **−11.428** | **0.000** | **−0.038** | **0.036** |
| 5. Experimental to positive control groups (11 weeks) (*H*, *L*) to (*I*, *M*), $n$ = 12 to 13 | **−182.194** | **0.000** | **1.048** | **0.019** | **0.037** | **0.005** |
| 6. Positive to negative control groups (11 weeks) (*I*, *M*) to (*G*, *K*), $n$ = 13 to 20 | **202.488** | **0.000** | **−12.476** | **0.000** | **−0.075** | **0.000** |
| Sex impact on offspring | | | | | | |
| 7. Female to male groups (3 weeks) (*D*, *E*, *F*) to (*A*, *B*, *C*), $n$ = 25 to 20 | 3.672 | 0.700 | 1.392 | 0.510 | 0.029 | 0.270 |
| 8. Female to male groups (11 weeks) (*K*, *L*, *M*) to (*G*, *H*, *I*), $n$ = 22 to 23 | −18.369 | 0.551 | −1.831 | 0.493 | 0.011 | 0.488 |

None of the effects are significant; nevertheless, $k_{G2}$ has the highest level of significance and will be considered for sex-specific classification in the second approach of the next section.

### *Classification of individual predictors*

An alternative to pooled predictor results can be obtained from the individual predictors, which are plotted in Fig. 5a for the subjects at 11 weeks of age in the three-dimensional predictor space for $\{G_{basal}, k_{G1}, k_{G2}\}$. At first glance, one can see a possible separation of clusters.

Based on these data, we automatically generated classification trees. As a first approach, a classification into three groups (negative control, experimental, and positive control) regarding maternal imprinting was performed. The obtained tree is shown in Fig. 5b. As a first criterion, subjects with $G_{basal} > 170$ will be assigned to the positive control group (= genetically determined); otherwise, they will be assigned to the negative control group (= healthy) in the case of $k_{G1} > 4.4$ yields; otherwise, they will be assigned to the experimental group. Apparently, $k_{G2}$ does not have an impact. As the confusion matrix in Fig. 5c shows, $P_c = 41/45 = 91.1\%$ of the subjects are classified correctly with these two simple rules (specificity regarding experimental group is $11/12 = 91.7\%$, sensitivity regarding negative control group is $17/20 = 85.0\%$).

As a second approach, we additionally distinguish male and female subjects, which leads to a total of six groups. The automatic generation results in a more complex classification tree, as shown in Fig. 5d. The most important predictors are still $G_{basal}$ and $k_{G1}$, although they have changed their order. Additionally, $k_{G2}$ comes into play, and it decides between sexes at the final stage. The confusion matrix in Fig. 5e proves that $P_c = 37/45 = 82.2\%$ of the subjects can still be classified correctly.

## Discussion

### *Proof of trans-generational diabetic programming*

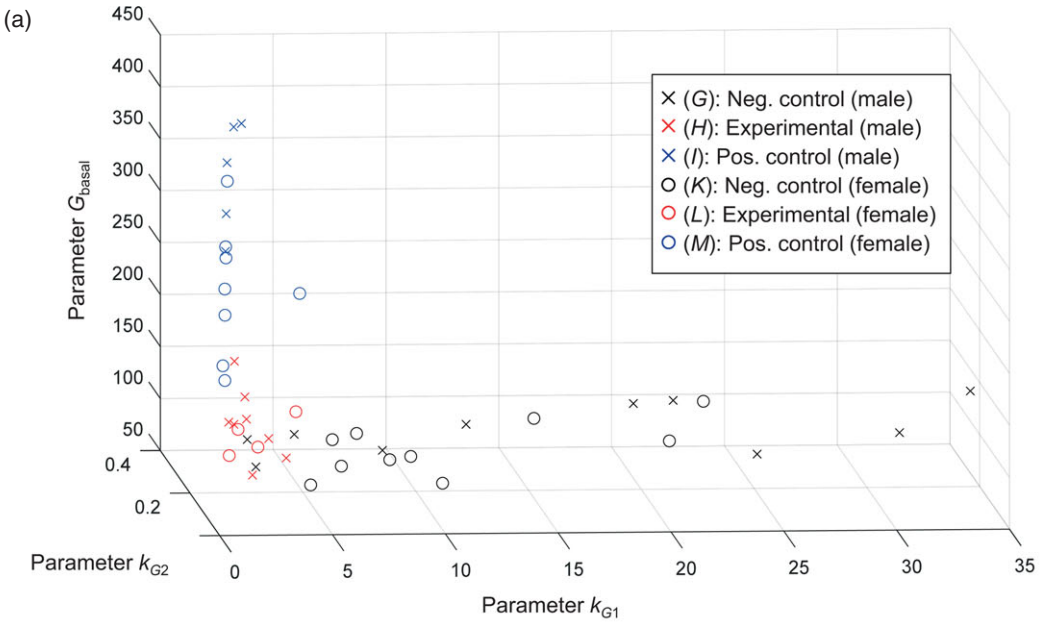
At 11 weeks of age, the experimental groups showed a specific metabolic behavior that differed significantly from both control groups. Mice of the negative control groups (*G*, *K*) and the experimental groups (*H*, *L*) are both not genetically programmed by Mendelian inheritance. Therefore, the reason for the different characteristics in the IPGTT and in the *in silico* model-based predictors must be located in the diabetic *in utero* milieu of the offspring. The difference between NDMs and DMs causes the observed trans-generational diabetic programming.

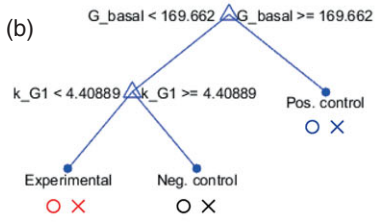
What are the underlying mechanisms? Experimental groups (*H*, *L*) did not show long-term glucose intolerances. The basal glucose levels as well as the levels after $t = 60$ min are in the physiological range specified by the control groups (*G*, *K*), see Fig. 4. This is confirmed by the predictors $G_{basal}$ as well as $k_{G2}$, respectively (see Table 2, line 4). Hence, glucose clearance or disposal follows different mechanisms compared to diabetic subjects in the positive control groups (*I*, *M*).

However, the short-term or first-phase response is disturbed (early peak in IPGTT in Fig. 4, decreased $k_{G1}$ in Table 2, line 4, and classification rule based on $k_{G1}$ in Fig. 5b). It is insulin mediated and may be caused by an altered reaction of the β-cells of the pancreas. Taking these observations together, we can conclude that the diabetic metabolism of mothers leads to altered insulin sensitivity as well as abnormal glucose-stimulated insulin release in offspring.

In the course of analyzing features of trans-generational diabetic programming in preliminary experiments, it could also be presented that early altered insulin sensitivity as well as abnormal glucose-stimulated insulin release is the part of diabetic malprogramming. Moreover, pancreases of wild-type offspring of

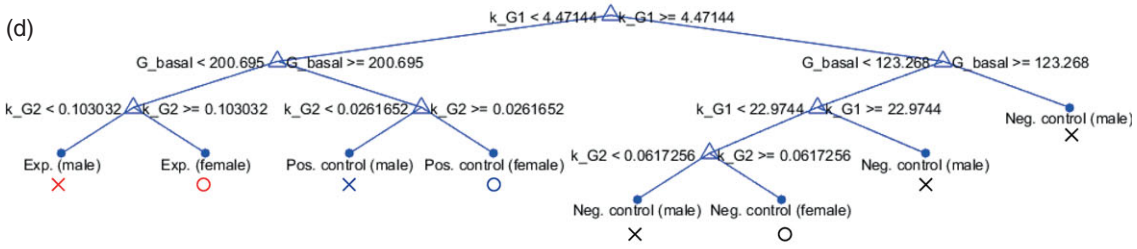
https://doi.org/10.1017/S2040174420000471 Published online by Cambridge University Press




(c)

| | | Predicted classes | | |
|---|---|---|---|---|
| | | Neg. control | Experimental | Pos. control |
| Known classes | Neg. control | **17** | **3** | 0 |
| | Experimental | 0 | **11** | **1** |
| | Pos. control | 0 | 0 | **13** |




(e)

| | | Predicted classes | | | | | |
|---|---|---|---|---|---|---|---|
| | | Neg. control (male) | Experimental (male) | Pos. control (male) | Neg. control (female) | Experimental (female) | Pos. control (female) |
| Known classes | Neg. control (male) | **7** | **3** | 0 | 0 | 0 | 0 |
| | Experimental (male) | 0 | **8** | 0 | 0 | 0 | 0 |
| | Pos. control (male) | 0 | 0 | **4** | 0 | 0 | **1** |
| | Neg. control (female) | **1** | 0 | 0 | **9** | 0 | 0 |
| | Experimental (female) | 0 | **2** | 0 | 0 | **2** | 0 |
| | Pos. control (female) | 0 | 0 | 0 | 0 | **1** | **7** |

**Fig. 5.** (a) Predictors $\{G_{basal}, k_{G1}, k_{G2}\}$ of $n = 45$ individual subjects of groups $(G) - (M)$ as marks in the predictor space, (b) classification tree for three groups with (c) corresponding confusion matrix, and (d) classification tree for six groups with (e) corresponding confusion matrix.

DMs showed a significantly lower pancreas mass and more uncoordinated islet organizations as well as morphological β-cell and islet alterations compared to the offspring of NDMs, which is in line with Reusens *et al.*[14]

The difference in programming in the experimental groups in relation to nondiabetic controls would remain undiscovered if only fasting glucose measurements (Fig. 4) were considered. To detect the altered insulin sensitivity as well as abnormal glucose-stimulated insulin release, the IPGTT glucose must be evaluated at $t = 15$, 30 min (Fig. 4), or even better the predictor $k_{G1}$ has to be assessed. This is clearly confirmed by the effect of predictor $k_{G1}$ in Table 2, line 4: by diabetic programming, a drop of –11.428 for $k_{G1}$ is predicted with high significance.

This observation is different from genetically programming by Mendelian inheritance, which can be easily detected by looking at fasting glucose levels in Fig. 4. The corresponding predictor $G_{basal}$ showed an effect of +202.488 mg/dl for groups $(I, M)$ compared to $(G, K)$ with high significance.

https://doi.org/10.1017/S2040174420000471 Published online by Cambridge University Press

### Impact of weaning

During the suckling period at 3 weeks of age, almost no significant differences between groups (*A*) – (*F*) were detected. Therefore, as already reported, suckling may have a protective effect targeting glucose homeostasis in offspring and mothers.[15] In Table 1, the mean values of the combined offspring groups during weaning can be compared with the later groups. Note that in the negative control groups, glucose tolerance is improved even after weaning. If we take this together with the previous section, diabetic programming receives verifiable effects during fetal stages but becomes apparent in offspring metabolism after weaning.[16]

### Sex-specific differences

Compared to the impacts of diabetic programming and weaning that were discussed before, sex-specific differences seem to be less dominant. No significant effects can be observed when combined female groups are compared to combined male groups, see Table 2, lines 7, 8. At 11 weeks of age, female plasma glucose levels $G$ in $t = 30$ and 45 min were lower than male levels (see Fig. 4). This observation is confirmed by the classification tree in Fig. 5d that uses $k_{G2}$ to distinguish the female and male groups. In general, the second-phase glucose tolerance seems to be advantageous in females compared to males.

## Conclusions

We show significant trans-generational impacts by analyzing diabetic programming patterns, which are clearly not genetically programmed by Mendelian inheritance (experimental group). These novel insights show that trans-generational diabetic programming leads to an evident change of patterns within the glucose insulin homeostasis in the next generations. The corresponding predictor $k_{G1}$ is highly significant.

The picture is clearly different from trans-generational genetically determined diabetes patterns (positive control group), which lead to significantly raised basal glucose values, as the predictor $G_{\text{basal}}$ shows.

Depending on both predictors $k_{G1}$ and $G_{\text{basal}}$ obtained from the individual *in silico* model of offspring, 91% of the subjects can be classified correctly with respect to maternal imprinting. We conclude, therefore, that maternal diabetes per se is a strong risk factor leading to alterations within glucose homeostasis and diabetes in their offspring early on.

The newly established dynamic *in vivo* and *in silico* models are suitable and highly sensitive for an individual early diagnosis of specific pathophysiological changes, trans-generational as well as for both generations. The model-based predictors proved to be appropriate to determine significant effects even for smaller group sizes and to successfully set up a diagnostic classification tree.

Moreover, our approach is well suited to diagnose pathophysiological changes early on to predict personalized developments and to start preventive therapies for both generations early[17] to assess specific maternal interventions, for example, a particular immunization as a possible therapeutic approach.[18]

**Acknowledgments.** The authors thank the teams of the Diabetes Center and Department of Internal Medicine IV of the Ludwig-Maximilians University of Munich (LMU) for support of the *in vivo* part of this work.

**Financial Support.** Partially funded by the "Förderprogramm für Forschung und Lehre (FöFoLe)" (projects 648/625 and 63/2009) of the Faculty of Medicine of the LMU, by the Friedrich-Baur-Stiftung (project 34/09), by the "Münchner Medizinische Wochenschrift (MMW) e.V.", and by the "Deutsche Diabetes Gesellschaft (DDG)".

**Conflicts of Interest.** None.

**Ethical standards.** The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national guides on the care and use of laboratory animals (German Animal Welfare Act) and has been approved by the Federal Government of Upper Bavaria.

## References

1. Guariguata L, Linnenkamp U, Beagley J, Whiting DR, Cho NH. Global estimates of the prevalence of hyperglycaemia in pregnancy. *Diabetes Res Clin Pract.* 2014; 103, 176–185. doi:10.1016/j.diabres.2013.11.003
2. IDF Diabetes Atlas. International Diabetes Federation, 9th edition, 2019. https://www.diabetesatlas.org/en/resources/
3. Eberle C, Ament C. Diabetic and metabolic programming – mechanisms altering the intrauterine milieue. *ISRN Pediatrics.* 2012; 2012, 975685. doi:10.5402/2012/975685
4. Eberle C, Palinski W, Ament C. A novel mathematical model detecting early individual changes of insulin resistance. *Diabetes Technol Ther.* 2013; 15, 870–880. doi:10.1089/dia.2013.0084
5. Eberle C, Niessen M, Hemmings BA, Tschopp O, Ament C. Novel individual metabolic profile characterizes the protein kinase B-alpha (pkbα-/-) in vivo model. *Arch Physiol Biochem.* 2014; 120, 91–98. doi:10.3109/13813455.2014.911330
6. Cobelli C, Dalla Man C, Sparacino G, Magni L, De Nicolao G, Kovatchev BP. Diabetes: models, signals, and control, methodological review . *IEEE Rev Biomed Eng.* 2009; 2, 54–96. doi:10.1109/RBME.2009.2036073
7. Bergman RN, Ider YZ, Bowden CR, Cobelli C. Quantitative estimation of insulin sensitivity. *Am J Physiol.* 1979; 236, E667–E677. doi:10.1152/ajpendo.1979.236.6.E667
8. Bergman RN, Phillips LS, Cobelli C. Physiologic evaluation of factors controlling glucose tolerance in man: measurement of insulin sensitivity and beta-cell glucose sensitivity from the response to intravenous glucose. *J Clin Invest.* 1981; 68, 1456–1467.
9. Eberle C, Ament C. Identifiability and online estimation of diagnostic parameters within the glucose insulin homeostasis. *Biosystems.* 2012; 107, 135–141. doi:10.1016/j.biosystems.2011.11.003
10. Eberle C, Ament C. Real-time state estimation and long-term model adaptation: a two-sided approach toward personalized diagnosis of glucose and insulin levels. *J Diabetes Sci Technol.* 2012; 6, 1148–1158. doi:10.1177/193229681200600520
11. Nelder JA, Mead R. A simplex method for function minimization. *Computer J.* 1965; 7, 308–312. doi:10.1093/comjnl/7.4.308
12. Montgomery DC. *Design and Analysis of Experiments*, 2019. John Wiley & Sons, New Jersey.
13. Coppersmith D, Hong SJ, Hosking JRM. Partitioning nominal attributes in decision trees. *Data Min Knowl Disc*, 1999; 3, 197–217. doi:10.1023/A:1009869804967
14. Reusens B, Ozanne SE, Remacle C. Fetal determinants of type 2 diabetes. *Curr Drug Targets.* 2007; 8, 935–941. doi:10.2174/138945007781386866
15. Martens PJ, Shafer LA, Dean HJ, *et al.* Breastfeeding initiation associated with reduced incidence of diabetes in mothers and offspring. *Obstet Gynecol.* 2016; 128, 1095–1104. doi:10.1097/AOG.0000000000001689
16. Garofano A, Czernichow P, Bréant B. In utero undernutrition impairs rat beta-cell development. *Diabetologia.* 1997; 40, 1231–1234. doi:10.1007/s001250050812
17. Smith RJ, Nathan DM, Arslanian SA, Groop L, Rizza RA, Rotter JI. Individualizing therapies in type 2 diabetes mellitus based on patient characteristics: what we know and what we need to know. *J Clin Endocrinol Metab.* 2010; 95, 1566–1574. doi:10.1210/jc.2009-1966
18. Eberle C, Merki E, Yamashita T, *et al.* Maternal immunization affects in utero programming of insulin resistance and type 2 diabetes. *PLoS One.* 2012; 7, e45361. doi:10.1371/journal.pone.0045361