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# Maternal diabetes causes coordinated down-regulation of genes involved with lipid metabolism in the murine fetal heart

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**Abstract**

Maternal diabetes is associated with increased transport of lipids to the fetus and increased risk of hypertrophic cardiomyopathy in the fetus. During fetal life, the heart normally has limited capacity to use lipids as fuel; and, at least in adults, cardiac lipid accumulation may lead to cardiomyopathy. Postnatally, lipid supply is increased when the offspring begins to suckle. We examined offspring from hypoinsulinemic $Ins2^{Akita}$ mice to assess whether maternal diabetes results in fetal myocardial hypertrophy and triglyceride accumulation and compared these with fetal hearts collected postnatally. On embryonic days 16 to 19, the fetal heart weight and triglyceride content were similar in offspring from $Ins2^{Akita}$ and nondiabetic wild-type mothers. The heart expression of lipid-metabolizing genes (peroxisomal proliferator-activated receptor α, lipoprotein lipase, fatty acid translocase, and fatty acid transport protein 1) was reduced in offspring from $Ins2^{Akita}$ mothers with high blood glucose levels and were closely intercorrelated, suggesting coordinated down-regulation. In contrast, on day 1 postnatally where the lipid availability to the heart is markedly increased, heart triglycerides and expression of several lipid-metabolizing genes (including lipoprotein lipase and fatty acid transport protein 1) were increased in offspring from wild-type mice. The results suggest that maternal type 1 diabetes mellitus in $Ins2^{Akita}$ mice does not cause cardiac hypertrophy or triglycerides accumulation in the fetal heart, possibly because of a coordinated down-regulation of genes controlling fatty acid uptake.


## 1. Introduction

The fetal heart uses mainly glucose and lactate rather than fatty acids as sources of adenosine triphosphate (ATP). Maternal diabetes causes increased transport of lipids from mother to fetus [1-7]. It is unknown how the heart may adapt to increased lipid availability during fetal life, but several different scenarios can be conceived. Immediately after birth, the heart increases its expression of several genes involved in triglyceride and fatty acid metabolism [8] in response to the increased fat availability during suckling. Hence, the fetal heart may change its substrate utilization in a pattern similar to that seen postnatally and start using fat as fuel instead of glucose. It is also possible, however, that the fetal heart stores the excess lipids as triglycerides or, yet again, to protect the heart against lipid accumulation and subsequently lipotoxicity, reduces its expression of genes responsible for lipid uptake into the cardiac myocytes [9]. Better understanding of fetal heart lipid metabolism during maternal diabetes may be clinically relevant. Infants of diabetic mothers have an increased risk of hypertrophic cardiomyopathy at birth. Hence, up to 40% of newborn infants of women with type 1 diabetes mellitus have cardiac enlargement with asymmetric septal hypertrophy [10-13]. Remarkably, the cardiac hypertrophy rapidly reverses postnatally, suggesting that the diabetic intrauterine environment is essential for the cardiac abnormality. Adult individuals with obesity and type 2 diabetes mellitus are at risk for developing a lipotoxic cardiomyopathy due to accumulation of lipids and by-products of lipid metabolism in the heart [9,14,15]. Thus, it is possible that if maternal diabetes causes lipid accumulation in the fetal heart, the hypertrophic cardiomyopathy in the infants might in fact be a transient fetal lipotoxic heart disease.

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In this study, we explored the effect of maternal diabetes on fetal heart hypertrophy and lipid metabolism by measuring heart weight, triglycerides, and expression of genes involved in lipoprotein lipolysis, fatty acid uptake, and $\beta$-oxidation in offspring of hypoinsulinemic Ins2$^{Akita}$ mice. The responses of the fetal heart to maternal diabetes were compared with the changes occurring postnatally in offspring from wild-type mice.

## 2. Methods and materials

### 2.1. Animals

Female Ins 2$^{Akita}$/+ (diabetic) mice and C57BL/6 control mice were obtained from Jackson Laboratories (Bar Harbor, ME). Male heterozygous human apolipoprotein B (apo B)–transgenic mice (B6.SJL-Tg [APOB100]) and male C57BL/6 control mice were obtained from M and B (Ry, Denmark). All mice were fed standard laboratory chow and housed in temperature-controlled facilities (21°C-23°C) with a 12-hour light, 12-hour dark cycle from 7:00 AM to 7:00 PM at the Panum Institute, Copenhagen University. The Animal Experiments Inspectorate, Ministry of Justice, Denmark, approved the study protocols.

### 2.2. Experimental protocol

The glucose concentration was determined in tail blood (after 4 hours of fasting) with a Glucometer Elite (Bayer, Lyngby, Denmark) before mating and immediately before removal of embryos. To see whether putative changes of maternal diabetes were accentuated by increasing the lipid transport to the fetus, the female Ins 2$^{Akita}$ mice were mated with heterozygous human apo B–transgenic male mice to create both wild-type and apo B–transgenic mice with diabetic mothers (assuming that the overexpression of the apo B transgene in the yolk sac [16] results in increased lipoprotein and lipid transport to the fetus). However, the fetal heart weight, triglyceride content, and expression of all heart genes examined were similar in human apo B–transgenic and wild-type fetuses. Therefore, data from wild-type and human apo B–transgenic offspring of diabetic mothers are presented together. The C57BL/6 control female mice were mated with C57BL/6 male mice.

Cesarean delivery was performed on days 16 to 19 of the pregnancies immediately after cervical dislocation of the mother. The fetuses were decapitated, and fetal hearts (without auriculae) and carcasses were immediately collected and placed in liquid $N_2$ and stored at −80°C until further analysis. Embryos with the Ins2$^{Akita}$ mutation were excluded from the study. To study the postnatal changes of myocardial triglycerides and gene expression, hearts and carcasses were collected from wild-type mice 1 day postnatally and processed similarly to the fetal tissue.

### 2.3. Genotyping of fetuses

Fetal carcasses were lysed by proteinase K; and subsequently, total cellular DNA was isolated using a silica-gel membrane (Dneasy kit; Qiagen, West Sussex, United Kingdom). Detection of a mutation in the insulin 2 gene (Ins 2$^{Akita}$) by restriction fragment length polymorphism was carried out as described previously [17]. The human apo B gene in apo B–transgenic fetuses was detected by polymerase chain reaction (PCR) using primers specific for human apo B (hApoBGT-51: 5′-cccagtatgttctccccaga-3′ and hApoBGT-31: 5′-tcagggtgtggagtttagcc-3′).

### 2.4. Cardiac triglycerides and free fatty acids

Lipids were extracted from fetal hearts with chloroform-methanol [18] and redissolved in toluol (1 $\mu$L/mg wet weight) for thin layer chromatography (TLC) [19]. The TLC plates (DC-fertigplatten SIL G-25; Macherey-Nagel, Duren, Germany; 20 × 20 cm) were impregnated with $Na_2$ EDTA (1 mmol/L, pH 5.5), dried, and washed in chloroform-methanol-water (60:40:10). The plates were activated at 110°C for 30 minutes, and 1-$\mu$L tissue extracts and triglyceride and free fatty acid standards (Sigma, Vallensbaek Strand, Denmark) with defined amounts of lipids were applied. The plates were developed in a 6-step procedure [19] before being placed in 10% cupric sulfate (wt/vol) in 8% phosphoric acid (vol/vol), dried, and baked for 2 minutes at 200°C. Triglycerides and free fatty acids in heart extract were quantified by digital image analysis. All samples were analyzed in duplicate on separate TLC plates. The precision and accuracy of this assay have been reported elsewhere [18,20].

### 2.5. Messenger RNA quantification

Total RNA was isolated from heart tissue with TriZol (Invitrogen, Taastrup, Denmark) in 1 mL Trizol (Invitrogen) per 100 mg frozen tissue. The RNA integrity was ensured with an RNA Nano LabChip (Agilent Technologies Denmark, Naerum, Denmark), and the RNA concentration was assessed from the absorbance at 260 nm. First-strand complementary DNA (cDNA) was synthesized from 1 $\mu$g of total RNA with Moloney murine leukemia virus reverse transcriptase (40 U; Roche, Avedore, Denmark) and random hexamer primers in 1-$\mu$L reactions. Primer pairs for mouse $\beta$-actin, mouse apo B, brain natriuretic peptide (BNP), carnitine palmitoyl-transferase (CPT) 1$\beta$, acyl coenzyme A: diacylglycerol acyltransferase (DGAT), fatty acid translocase (FAT/CD36), fatty acid transport proteins 1 and 4 (FATP1 and FATP4), heart-specific fatty acid binding protein (hFABP), long-chain acyl–coenzyme A dehydrogenase (LCAD), lipoprotein lipase (LPL), microsomal triglyceride transfer protein (MTP), and peroxisomal proliferator-activated receptor (PPAR) $\alpha$ have been reported elsewhere [18,20,21]. The primers for mouse PPAR$\gamma$-coactivator 1$\alpha$ (PGC-1$\alpha$-51: 5′-ggcacatctgttcttccaca-3′ and PGC-1$\alpha$-31:

5′-gccatcccttagttcactgg-3′) were obtained from Sigma-Genosys (Pampisford, United Kingdom).

The specificity of each PCR was confirmed by DNA sequencing of upper and lower strands of reverse transcriptase–PCR transcripts. Real-time PCR analyses were done with a LightCycler and a FAST START DNAmaster SYBR Green kit (Roche, Hvidovre, Denmark). The PCR reactions (20 $\mu$L) contained 2 $\mu$L of SYBR Green I mixture, 2 to 3 mmol/L $MgCl_2$, 10 pmol of each primer, cDNA synthesized from 2 ng of total RNA, and PCR-grade $H_2O$. The relationship between the time point of the log-linear increase of the fluorescence signal and the concentration of a messenger RNA (mRNA) transcript was determined in each run by coanalyzing a dilution series of mouse liver cDNA made from 10, 2, 0.2, and 0.02 ng of total RNA.

### 2.6. Statistics

Data are presented as mean with SEM. Comparisons between groups were performed with analysis of variance or *t* test when appropriate.

## 3. Results

To study the effect of maternal diabetes on the embryonic heart, we used $Ins2^{Akita}$ female mice that are hyperglycemic and hypoinsulinemic as a result of a mutation in one of the insulin genes causing misfolding of the insulin protein and, subsequently, deficient insulin secretion [22]. The fasting blood glucose concentration was markedly higher in $Ins2^{Akita}$ mice than in wild-type controls before they became pregnant (Fig. 1A). Similarly to women with type 1 diabetes mellitus in which the fasting blood glucose levels decrease from week 7 through week 15 of gestation [23], the hyperglycemic effect of the $Ins2^{Akita}$ mutation was reduced during pregnancy (Fig. 1A). When the fetal hearts were removed on embryonic days 16 to 19, only ~50% of the $Ins2^{Akita}$ mothers had blood glucose concentrations above the levels of the control group (Fig. 1A). Hence, data from offspring of $Ins2^{Akita}$ mothers with blood glucose concentrations below or above the median were analyzed separately. To avoid potential confounding effects of defective insulin secretion in the offspring, we initially genotyped each offspring and excluded those that had inherited the $Ins2^{Akita}$ mutation from their mother.

Diabetes increases cardiac triglyceride stores in rodents [24-26]. However, when we examined fetal cardiac triglycerides in offspring from $Ins2^{Akita}$ and wild-type mothers with a sensitive TLC-based method, the average triglyceride concentrations were similar in the groups (Fig. 1B), as were the average concentrations of free fatty acids (3.4 ± 0.5, 3.6 ± 0.5, and 3.8 ± 0.5 nmol/mg wet weight in offspring of wild-type mothers, offspring of $Ins2^{Akita}$/+ mothers with low blood glucose values, and offspring of $Ins2^{Akita}$/+ mothers with high blood glucose values, respectively). In contrast, the average heart triglyceride concentration increased 72% in pups 1 day old (Fig. 1C).

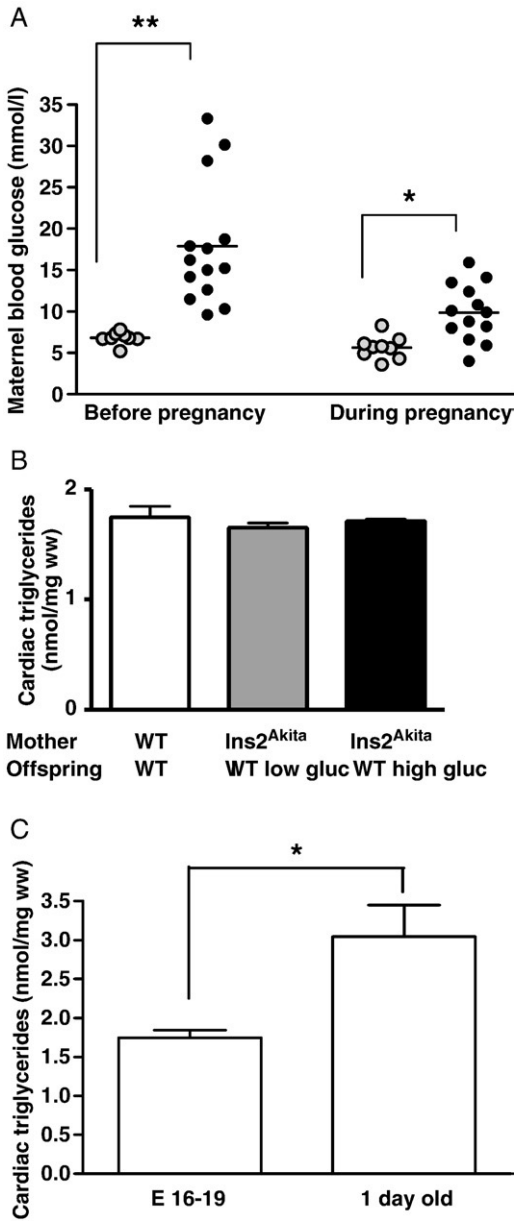


Fig. 1. A, Blood glucose concentrations in female $Ins2^{Akita}$/+ mice (n = 14, black dots) and controls (n = 9, grey dots) before and during pregnancy. Results were analyzed with an unpaired *t* test with Welch correction. **P* = .001 and ***P* = .0001. The lines depict mean. B, Fetal cardiac triglyceride content in offspring of wild-type mothers (n = 11) and in offspring of $Ins2^{Akita}$/+ mothers (n = 14) (all samples collected at embryonic days 16-19). Values from offspring of $Ins2^{Akita}$/+ mothers were divided into 2 groups by the median of the maternal blood glucose values late in gestation: maternal blood glucose ranging from 4.0 to 8.2 mmol/L (low glucose) and from 8.8 to 15.9 mmol/L (high glucose). Each female provided 1 to 2 pups. C, Fetal cardiac triglyceride content in offspring of wild-type mothers at embryonic days 16 to 19 (1.75 ± 0.33 nmol/mg ww, n = 11) and 24 hours after delivery (3.01 ± 0.70 nmol/mg ww, n = 3). Results were analyzed with Mann Whitney *U* test. **P* = .02. E 16-19 indicates embryonic days 16 to 19; WT, wild type.

We next assessed the impact of maternal diabetes in $Ins2^{Akita}$ mice on expression of genes involved in triglyceride metabolism in the fetal heart. The mRNA expressions of

PGC-1α, PPAR-α, LPL, FAT/CD36, and FATP1 were significantly decreased in the offspring from Ins2$^{Akita}$ mothers with elevated blood glucose concentration (Fig. 2A). The heart mRNA levels of FATP4 and hFABP mRNA also tended to be reduced by maternal diabetes, although these effects were not statistically significant, whereas the heart mRNA expressions of CPT1, LCAD, DGAT, apo B, and MTP were similar in the 3 groups (Fig. 2A). Accordingly, the fetal heart mRNA expressions of PGC-1α, PPAR-α, LPL, FAT/CD36, FATP1, and FATP4 displayed strong negative correlation with the maternal blood glucose concentration (PGC-1α, Pearson $r = -0.61$, $P = .01$; PPAR-α, $r = -0.80$, $P = .0002$; LPL, $r = -0.70$, $P < .004$; FAT/CD36, $r = -0.77$, $P < .0005$; and FATP1, $r = -0.61$, $P < .02$). There were no differences in the heart gene expression levels between offspring from Ins2$^{Akita}$ mothers with low blood glucose concentrations and offspring from wild-type mothers (Fig. 2A).

Within fetuses from Ins2$^{Akita}$ mice, the heart mRNA expression of PGC-1α, PPAR-α, LPL, FATP1, FAT/CD36, and hFABP mRNA correlated significantly with each other (Table 1). The LCAD, DGAT, and CPT1 as well as apo B and MTP mRNA levels also correlated with each other but not with the aforementioned genes. This suggests that the changes in the expression of lipid-metabolizing genes in the fetal heart caused by maternal diabetes are highly coordinated. To examine whether the changes in lipid-metabolizing genes occurring postnatally are coordinated in a similar fashion, we examined hearts from 1-day–old wild-type mice. Opposite to the observations in offspring of diabetic mothers, the heart mRNA expression of PGC-1α, LPL, FATP1, and LCAD increased 1 day postnatally

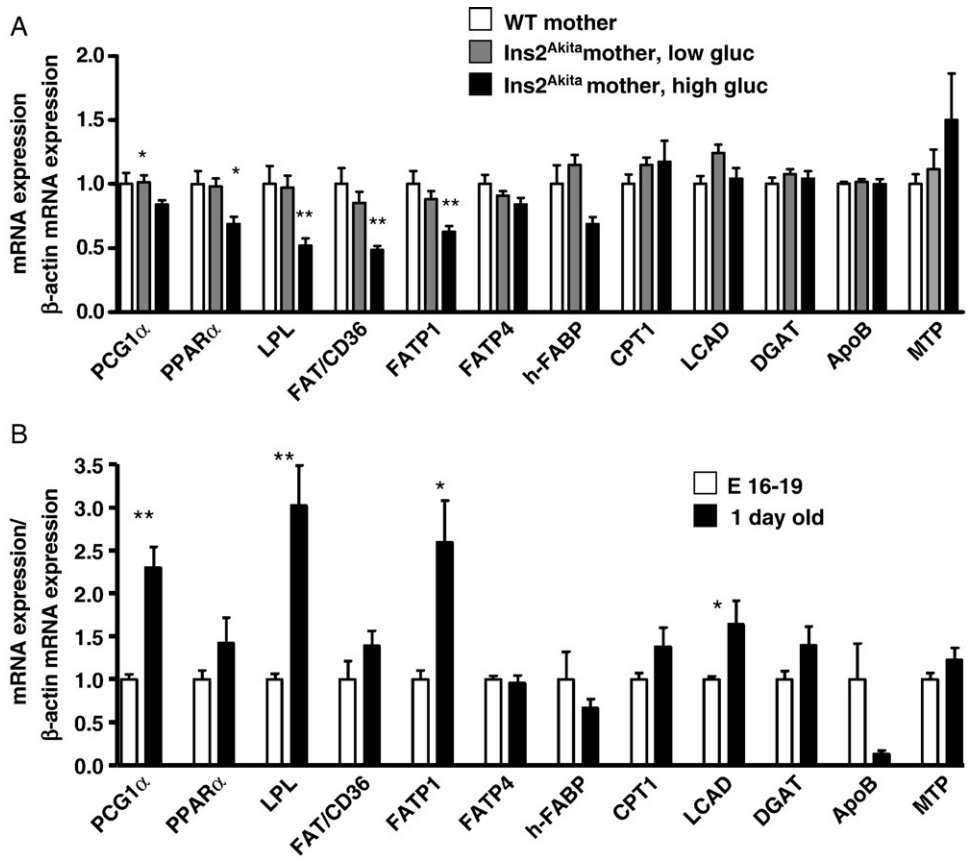


Fig. 2. A, Heart expression of genes affecting cardiac fatty acid metabolism and lipoprotein synthesis. The mRNA contents of the indicated genes were measured by real-time PCR in fetal hearts collected from embryos on days 16 to 19 of pregnancy from offspring of wild-type mothers (n = 8–9) and offspring of Ins2$^{Akita}$/+ mothers (n = 15–16) (wild-type offspring, n = 7–8; apo B–transgenic offspring, n = 7–8). Values from offspring of Ins2$^{Akita}$/+ mothers were divided into 2 groups (n = 8 in both groups) by the median of the maternal blood glucose values late in gestation: maternal blood glucose ranging from 4.0 to 8.2 mmol/L (grey bars) and from 8.8 to 15.9 mmol/L (black bars). Each female provided 1 or 2 pups. In each sample, the mRNA expression was normalized with the β-actin mRNA content. Expression in samples from offspring of Ins2$^{Akita}$/+ mothers was normalized to expression in samples from offspring of wild-type mothers. *$P < .05$. **$P < .02$. B, Heart expression of genes involved in cardiac fatty acid metabolism and lipoprotein synthesis. The mRNA contents of the indicated genes were measured by real-time PCR in fetal hearts collected from embryos on days 16 to 19 of pregnancy (white bars, n = 9) and from pups 1 day after delivery (black bars, n = 9). In each sample, the mRNA expression was normalized with the β-actin mRNA content. Expression in samples from pups 1 day old was normalized to expression in samples from embryos on days 16 to 19. *$P < .05$ and **$P < .001$. Results were analyzed with unpaired $t$ test and corrected with Welch correction when appropriate.

Table 1
Correlation coefficients based on data from gene expression quantitation by real-time PCR from fetal hearts from offspring of $Ins2^{Akita}$/+ mothers

| | *PCG1-α* | *PPAR-α* | *LPL* | *FATP1* | *FATP4* | *FAT/CD36* | *H-FABP* | *CPT1* | *LCAD* | *DGAT* | *ApoB* | *MTP* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PCG1-α | 1.00 | | | | | | | | | | | |
| PPAR-α | 0.68 | 1.00 | | | | | | | | | | |
| LPL | 0.79 | 0.83 | 1.00 | | | | | | | | | |
| FATP1 | 0.61 | 0.64 | 0.91 | 1.00 | | | | | | | | |
| FATP4 | 0.09 | 0.31 | 0.50 | 0.60 | 1.00 | | | | | | | |
| FAT/CD36 | 0.77 | 0.81 | 0.85 | 0.75 | 0.32 | 1.00 | | | | | | |
| H-FABP | 0.64 | 0.64 | 0.76 | 0.69 | 0.24 | 0.89 | 1.00 | | | | | |
| CPT1 | -0.43 | -0.14 | -0.24 | -0.19 | -0.07 | -0.14 | 0.12 | 1.00 | | | | |
| LCAD | 0.00 | 0.15 | 0.30 | 0.39 | 0.21 | 0.17 | 0.44 | 0.69 | 1.00 | | | |
| DGAT | -0.20 | -0.02 | -0.07 | -0.10 | 0.06 | 0.09 | 0.39 | 0.55 | 0.45 | 1.00 | | |
| ApoB | 0.22 | 0.10 | 0.24 | 0.19 | 0.24 | 0.39 | 0.35 | -0.02 | 0.07 | 0.34 | 1.00 | |
| MTP | -0.28 | 0.01 | -0.20 | -0.18 | 0.23 | -0.41 | -0.51 | 0.14 | 0.06 | -0.48 | -0.78 | 1.00 |

All correlation coefficients above 0.50 (n = 16) are significant with $P < .05$ (shaded areas).

(Fig. 2B). The heart mRNA expression of PGC-1α, PPAR-α, and LPL, as well as that of FAT/CD36, hFABP, CPT1, LCAD, and DGAT, were correlated with each other in the postnatal hearts (Table 2).

Maternal diabetes can cause fetal hypertrophic cardiomyopathy in humans [10-13] and increases the size of the fetal heart in rats with streptozotocin-induced hypoinsulinemic diabetes [27-29]. However, the heart-to–body weight ratio was not increased in offspring from $Ins2^{Akita}$ mothers with hyperglycemia compared with controls (Fig. 3A). Cardiac hypertrophy in humans is associated with increased expression of BNP in the heart, and the cord blood concentration of BNP is increased in newborns when the mothers have type 1 diabetes mellitus [30]. Furthermore, cardiac BNP was increased in offspring from female rats with streptozotocin-induced hypoinsulinemic diabetes [29]. Surprisingly, however, the cardiac BNP mRNA expression was decreased in offspring from $Ins2^{Akita}$ mothers (Fig. 3B); and the BNP mRNA expression was inversely associated with the maternal blood glucose concentration ($r = -0.76$, $P = .0006$; data not shown).

## 4. Discussion

The main finding of the present study is that maternal diabetes in $Ins2^{Akita}$ mice down-regulates expression of genes that govern cardiac lipid uptake in the fetal heart (ie, PGC-1α, PPAR-α, LPL, FAT/CD36, and FATP1). PGC-1α and PPAR-α are major regulators of myocardial lipid metabolism [31,32], LPL generates free fatty acids by lipolysis of lipoprotein triglycerides in the myocardial capillaries [33], and FAT/CD36 and FATP1 [34-36] are involved with fatty acid transport across the myocyte cell membrane. The expression of the individual genes correlated closely with the maternal blood glucose concentration and with each other, suggesting that the fuel utilization of the fetal heart is affected in a coordinated manner by maternal diabetes.

The LPL, FAT/CD36, and FATP1 are all target genes of PPAR-α. Nevertheless, other PPAR-α target genes in the myocardium, such as hFABP, CPT1, and LCAD, are not affected by the change in PPAR-α by maternal diabetes. Hence, other factors than PPAR-α must also be important for the regulation of fetal myocardial lipid metabolism in a

Table 2
Correlation coefficients based on data from gene expression quantitation by real-time PCR from fetal hearts collected from embryos on postnatal day 1

| | PCGIa | PPARa | LPL | FATP1 | FATP4 | FAT/CD36 | H-FABP | CPT1 | LCAD | DGAT | ApoB | MTP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FCGIa | 1.00 | | | | | | | | | | | |
| PPARa | 0.99 | 1.00 | | | | | | | | | | |
| LPL | 0.93 | 0.89 | 1.00 | | | | | | | | | |
| FATP1 | 0.46 | 0.45 | 0.46 | 1.00 | | | | | | | | |
| FATP4 | 0.46 | 0.45 | 0.46 | 1.00 | 1.00 | | | | | | | |
| FAT/CD36 | 0.41 | 0.35 | 0.50 | 0.88 | 0.88 | 1.00 | | | | | | |
| H-FABP | 0.67 | 0.64 | 0.71 | 0.90 | 0.90 | 0.94 | 1.00 | | | | | |
| CPT1 | 0.82 | 0.79 | 0.87 | 0.82 | 0.82 | 0.81 | 0.95 | 1.00 | | | | |
| LCAD | 0.78 | 0.76 | 0.80 | 0.87 | 0.87 | 0.82 | 0.95 | 0.99 | 1.00 | | | |
| DGAT | 0.73 | 0.74 | 0.69 | 0.80 | 0.80 | 0.53 | 0.73 | 0.84 | 0.88 | 1.00 | | |
| ApoB | -0.17 | -0.18 | -0.16 | 0.13 | 0.13 | -0.01 | -0.08 | -0.10 | -0.07 | -0.03 | 1.00 | |
| MTP | 0.36 | 0.37 | 0.32 | 0.90 | 0.90 | 0.85 | 0.83 | 0.67 | 0.72 | 0.57 | 0.20 | 1.00 |

All correlation coefficients of 0.67 or above (n = 9) are significant with $P < .05$ (shaded areas).

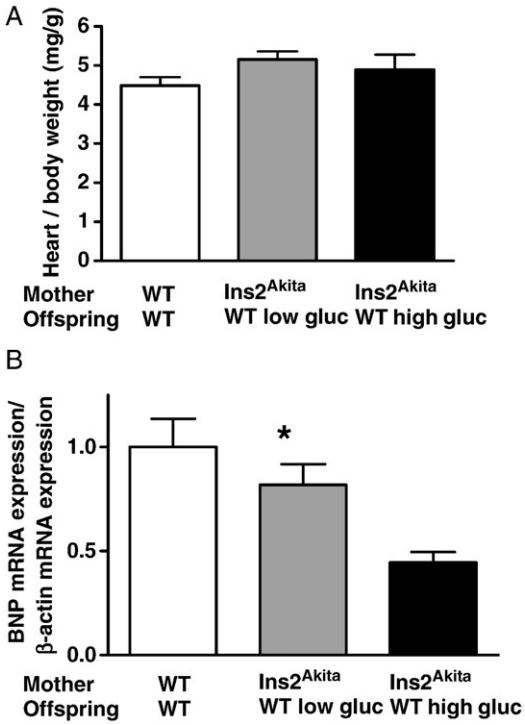


Fig. 3. A, Fetal heart-to–body weight ratio in offspring of wild-type mothers (n = 19) and in offspring of $Ins2^{Akita}$/+ mothers (n = 32) (wild-type offspring, n = 16; apo B–transgenic offspring, n = 16) (all samples collected at embryonic days 16-19). Values from offspring of $Ins2^{Akita}$/+ mothers were divided into 2 groups by the median of the maternal blood glucose values late in gestation: maternal blood glucose ranging from 4.0 to 8.2 mmol/L (low glucose, grey bar) and from 8.8 to 15.9 mmol/l (high glucose, black bar). B, The mRNA expression of mouse BNP in myocardium from wild-type offspring of wild-type mothers (n = 9) and from offspring of mothers with diabetes (Ins $2^{Akita}$/+) (wild-type offspring, n = 8; apo B–transgenic offspring, n = 8). Values from offspring of $Ins2^{Akita}$/+ mothers were divided into 2 groups by the median of the maternal blood glucose values late in gestation: maternal blood glucose ranging from 4.0 to 8.2 mmol/L (low glucose, grey bars) and from 8.8 to 15.9 mmol/L (high glucose, black bars). Each female provided 1 or 2 pups. In each sample, the mRNA expression was normalized with the $\beta$-actin mRNA content. Results were analyzed with an analysis of variance. $*P < .05$.

diabetic intrauterine environment. Furthermore, the changes in lipid handling in the postnatal heart may also involve posttranslational changes in protein expression and cellular localization. For instance, angiopoietin-like protein 4 (which is subject to rapid changes in the postprandial state) converts the catalytically active, dimeric form of LPL to catalytically inactive monomers [37]; and insulin induces the translocation of intracellular FAT/CD36 and FATP1/4 to the plasma membrane, thereby increasing cellular fatty acid uptake [38,39].

The transport of free fatty acid to the fetus is increased by maternal diabetes in several species [3-5,7]. This increased exposure of the fetal heart to lipids maybe similar to the situation postnatally, when large amounts of fat are supplied with the milk during suckling. Nevertheless, the effects of maternal diabetes on heart expression of genes affecting lipid uptake and oxidation were distinct from those seen postnatally where the expression of PGC-1$\alpha$, LPL, FATP1, and LCAD (involved with $\beta$-oxidation of fatty acids) increased. Postnatally, increased plasma levels of free fatty acids suppressing glucose utilization, hormonal changes with decreased insulin, and increased glucagon and thyroid hormones as well as the increase in blood oxygen tension have been suggested to affect substrate utilization [40].

Furthermore, the effect of maternal diabetes on fetal heart gene expression is opposite to that seen in adult diabetes where glucose utilization was suppressed and fatty acid oxidation increased [21,41]. In the adult diabetic heart, the elevated levels of free fatty acids caused by increased lipolysis of triglyceride stores in peripheral adipose tissues as well as within the cardiac capillaries are thought to be a major causing factor in the “metabolic inflexibility” of the diabetic heart [42,43].

In addition to increasing free fatty acid delivery to the fetus, maternal diabetes causes fetal hyperinsulinemia [44]. This may increase glucose and reduce fatty acid utilization in the fetal heart. However, it is unknown whether hyperinsulinemia also results in reduced expression of lipid-metabolizing genes in the normal fetal heart. Unlike the adult diabetic heart, where the increased fatty acid levels result in accumulation of neutral fat in the cardiac myocytes, and unlike the postnatal heart, which also displayed increased triglyceride storage in the present study, fetal heart triglyceride stores were not affected by maternal diabetes in $Ins2^{Akita}$ mice.

The perinatal changes in myocardial metabolism have been investigated in several species. The combined evidence suggests that during fetal life, the myocardium uses mainly glucose and lactate, whereas after birth, the myocardium relies on fatty oxidation as a source of ATP [40]. This change occurs with the beginning of the suckling period where the intake of the maternal milk rich in triglycerides results in increased levels of circulating fatty acid in the newborn compared with the fetus [45]. Induction of myocardial fatty acid oxidation in rabbits occurs not immediately after birth but is apparent after 7 days [46,47]. In the present study, where we collected the heart in 1-day–old pups, the expression of LPL and FATP1 was increased to a much higher degree than that of LCAD. Thus, increased supply of fatty acids to the myocardium at a time where fatty acid oxidation is not yet at its highest might explain the accumulation of triglycerides in the hearts of the 1-day–old pups.

Changes in the expression of LPL, FAT/CD36, and FATP1 affect lipid uptake by the heart in mice [48-50]. The lack of triglyceride accumulation in the fetal heart in offspring of diabetic mothers despite increased free fatty acid availability might therefore at least in part reflect a protective response of the fetal heart to maternal diabetes by decreased expression of LPL, FAT/CD36, and FATP1. Similarly, a decrease in myocardial PPAR-$\alpha$ mRNA expression was found in rats when supply of free fatty acid was increased by inducing diabetes (streptozotocin), by fasting, or by high-fat feeding [51].

The expression of PGC-1α together with other lipid-metabolizing genes is decreased in pathologic cardiac hypertrophy in the mouse [8,31,52]. Thus, diabetes in $Ins2^{Akita}$ mothers conferred changes in the fetal heart gene expression similar to those occurring during development of hypertrophic cardiomyopathy. Myocardial hypertrophy develops in offspring from streptozotocin-treated rats with hypoinsulinemic diabetes [27-29]; and in humans, the plasma BNP concentration is increased in the newborn when the mother has type 1 diabetes mellitus [30]. Brain natriuretic peptide is a molecular marker of cardiac hypertrophy [53]. Therefore, we anticipated that the offspring from the diabetic $Ins2^{Akita}$ mothers would display myocardial hypertrophy and an accompanying increase of the heart BNP mRNA expression. However, the fetal heart weight was not affected and the heart BNP mRNA expression was decreased in the offspring from $Ins2^{Akita}$ mothers. The apparent differences between humans, streptozotocin-treated rats, and $Ins2^{Akita}$ mice may to some extent be due to species differences. It should be noted that hyperglycemia can induce cellular apoptosis [54] and attenuates the expression of antiapoptotic proteins in the heart of adult rats [55]. Hence, hyperglycemia-induced increases in apoptosis rates in the offspring of $Ins2^{Akita}$ mothers as such may have attenuated the hypertrophic effect of maternal diabetes.

The magnitude of hyperglycemia in $Ins2^{Akita}$ mice was blunted during pregnancy. In humans, hypoglycemic events in first-trimester type 1 diabetes mellitus patients were first described more than 50 years ago [56]. In normal pregnancies, plasma glucose decreases between weeks 6 and 10 of gestation [57]. In type 1 diabetes mellitus pregnancies, the decrease is mainly seen from week 7 through week 15 of gestation [23]. This antidiabetic effect of pregnancy may be due to increased insulin sensitivity [58,59] reflecting the decrease of the anti-insulin hormone progesterone [23] and the increase of 17-estradiol (which stimulates expression of insulin receptors [60]). The $Ins2^{Akita}$ mouse may be a valuable model for future studies to dissect such mechanisms.

In summary, the present study demonstrates that maternal diabetes in $Ins2^{Akita}$ mice causes a coordinated down-regulation of genes affecting lipid uptake in the fetal heart without affecting cardiac triglyceride stores or causing cardiac hypertrophy. Further studies delineating any long-term effect of maternal diabetes on the myocardium of the offspring postnatally are needed.

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