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Postnatal Treatment With Dexamethasone Perturbs Hepatic and Cardiac Energy Metabolism and Is Associated With a Sustained Atherogenic Plasma Lipid Profile in Suckling Rats

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ABSTRACT: Early exposure to glucocorticoids (GC) has been proposed to disturb hepatic and cardiac function in later life. In the present study, we evaluated early metabolic alterations upon GC treatment that may predispose to long-term abnormalities. Rats were injected with dexamethasone (DEX) at d 1, 2, and 3 after birth and controls received saline (SAL). Rats were killed at 2, 7, and 14 d of age. Compared with SAL, DEX induced lower plasma insulin levels, hyperglycemia, hyperketonemia, and dyslipidemia at 2 d. At the same time, DEX treatment significantly increased expression of gluconeogenic and fatty acid oxidation genes in liver and expression of genes involved fatty acid utilization in heart. At 7 d, DEX-treated rats showed insulin resistance with hyperlipidemia, whereas hepatic and cardiac gene expression patterns were largely normalized. Hyperlipidemia and a significantly increased hepatic triglyceride content in DEX-treated rats were prominent at 14 d without large differences in hepatic and cardiac gene expression patterns. Thus, neonatal DEX administration transiently affects cardiac and hepatic gene expression patterns in suckling rats associated with sustained effects on plasma glucose and lipid concentrations. Whether these early effects of DEX contribute to hepatic and cardiac abnormalities at adult age needs further evaluation. **(*Pediatr Res* 61: 165–170, 2007)**

GC are used to prevent chronic lung disease in immature newborns because of their anti-inflammatory action. However, despite the evident short-term benefit, concern has emerged about potential long-term negative consequences (1,2). Cardiovascular disease is one of the negative consequences proposed to occur after neonatal DEX administration in preterm human newborns, including transient cardiac hypertrophy (3) and hypertension (4). Recent studies conducted in rats have revealed that neonatal DEX administration leads to cardiac dilatation and reduced systolic function at 4 wk (5) and to cardiac hypertrophy at 45 wk of age (6). So far, the molecular mechanism by which postnatal administration of GC induces cardiac dysfunction later in life has remained unknown.

Epidemiologic studies have raised the "fetal origins of adult disease" hypothesis, stating that early lifestyle factors, imposed by intrauterine or neonatal environment, increase the risk of developing cardiovascular disease and hypertension in later life (7). In particular, exposure to excess GC during critical early life stages has been implicated as a factor that may program long-term changes in cardiac function, predisposing to adult disease (8).

The heart requires a continuous energy supply to maintain its contractile function, while its capacity to store energy-rich substrates is limited. Under resting conditions, the adult heart derives about 70% of its energy from lipid oxidation and the remainder from glucose (9). Several recent studies have demonstrated that altered cardiac energy utilization might be a cause for cardiomyopathy (10,11). However, whether cardiac genes involved in energy metabolism are persistently affected during that process has not been reported.

GC administration leads to insulin resistance, hyperglycemia, and dyslipidemia in preterm babies (12) and in experimental animals (13). The liver performs a crucial role in maintaining lipid and glucose homeostasis. GC administration may lead to hepatic insulin resistance, contributing to hyperglycemia as well as dyslipidemia in rats (14). Data accumulated in recent years have indicated that GC exposure during early life may cause liver dysfunction in later life, contributing to metabolic derangements (8,15) and increased risk for cardiovascular disease. Therefore, neonatal GC administration may disturb hepatic energy metabolism with a consequently unbalanced cardiac energy supply. However, available data on hepatic energy metabolism, especially gene expression at neonatal stage, are still quite limited.

In the present study, we investigated the short-term effect of GC, particularly of DEX, on hepatic and cardiac expression of genes involved in energy metabolism in relation to plasma concentrations of metabolic substrates in suckling rats, trying

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Abbreviations: ANF, atrial natriuretic peptide; **CPT,** carnitine palmitoyltransferase; **DEX,** dexamethasone; **G6PHT,** glucose-6-phosphatase transport protein 1; **GC,** glucocorticoids; **PEPCK,** phosphoenolpyruvate carboxykinase 1, cytosolic; **PPAR,** peroxisome proliferator-activated receptor; **SAL,** saline; **TnnT2,** troponin T; **UCP-2 and 3,** uncoupling protein 2 and 3

to identify early signs that may predispose to heart disease in later life.

MATERIALS AND METHODS

Animals. Pregnant Wistar rats (270–300 g) were housed individually and kept under conventional housing conditions with free access to food and water. Pups were born naturally on d 21–22 of gestation. On the day of birth (d 0), male pups were selected and randomly divided into treatment or control groups fed by "foster mothers" (six pups per litter). Temperature and humidity were kept constant and the dams had free access to food and water. An artificial 12-h light/12-h dark cycle was used. Pups in the treatment group were injected intraperitoneally with DEX 21-phosphate at d 1, 2, and 3 (0.5, 0.3, and 0.1 μg/g body weight, respectively) after birth in the morning from 0900 to 1000 h. Controls were injected with equal volumes (10 μL/g) of sterile pyrogen-free SAL. Pups were killed by decapitation at 2 , 7, and 14 d of age after birth (n = 11–12 per group) after samples of blood were collected from the abdominal artery under anaesthesia between 0900 and 1200 h.

All experimental procedures were approved by the local ethical committee for animal experiments.

Analysis. Blood glucose was measured by the Medisense Precision glucose meter (Medisense Precision, Abbott Laboratories, Abbott Park, IL) immediately after blood sampling. The remainder was centrifuged and plasma was separated and stored at –20°C until analysis. Plasma concentrations of total and free cholesterol and of triglycerides were measured with commercially available kits, as previously described (16). Pooled plasma samples from 10 rat pups per treatment group per age were used for lipoprotein separation by fast protein liquid chromatography (FPLC) (17). Plasma insulin concentration was measured by Luminex-based bead array method using LINCOplex simultaneous multi-analyte detection system (Millipore, Billerica, MA) following the manufacturer's instructions. Plasma concentrations of ketone bodies were measured by commercially available kits on a Selectra 2 auto analyzer (Merck, Darmstadt, Germany). Hepatic lipid content was measured as previously described (16) after Bligh and Dyer (18) extraction.

Morphologic analysis. Liver and heart were examined after periodic acid–Schiff (PAS) staining for glycogen (n = 6 per group) and Oil-red-O (ORO) staining for neutral lipids (n = 4 per group). Hematoxylin-eosin (H&E) staining was used for heart morphology (n = 6 per group). H&E and PAS staining were performed on 4% formalin-fixed, paraffin-embedded sections by standard procedures. ORO staining was performed on frozen sections by standard procedures.

RNA isolation and quantitative real-time PCR. Heart and liver tissues used for RNA isolation were rapidly excised and frozen in liquid nitrogen (n = 11–12 each group). RNA was extracted from heart and liver samples by using TRI reagent (Sigma Chemical Co., St. Louis, MO). The integrity of RNA was assessed using Lab-on-a-Chip 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). Total RNA was reverse transcribed as described previously (19). Real-time quantitative PCR was performed using an ABI PRISM 7700 sequence detector (Applied Biosystems, Foster City, CA). Primers were obtained from Invitrogen (Carlsbad, CA). Fluorogenic probes, labeled with 6-carboxy-fluorescein and 6-carboxy-tetramethyl-rhodamine, were made by Eurogentec (Seraing, Belgium). Primer sequences are listed in Table 1 (See supplemental material online) and published sequences are listed on our web site at www.labpediatricsrug.nl. Heart gene expressions were standardized by "normalization factors" calculated by GeNorm (20) software from the following house keeping genes: β-actin, 18S, 36B4, and cyclophilin, because none of these genes alone was sufficiently stable as standard calculated by GeNorm. All hepatic gene expression data were standardized to β-actin mRNA.

Data analysis. Data were expressed as mean ± SD. Statistical analysis of groups was assessed by t test or Mann–Whitney U test. Differences within group were assessed by one-way ANOVA. Level of significance was set at $p < 0.05$. Analyses were performed using SPSS for Windows software (SPSS, Chicago, IL).

RESULTS

Neonatal DEX treatment leads to growth retardation in suckling rats. Compared with SAL-treatment, neonatal DEX administration led to a significantly reduced body weight gain, which was manifest already at 24 h after initiation of DEX treatment. A significantly lower body weight was found during the entire experimental period (2–14 d of age) (Fig. 1). A significantly lower heart weight was noted following DEX administration at all ages (Table 2). A significantly higher heart weight/body weight ratio was, however, found only at 7 d old rats upon DEX treatment, suggesting transient cardiac hypertrophy at that age. Neonatal DEX treatment led to a significantly higher liver weight/body weight ratio at 2 d of age.

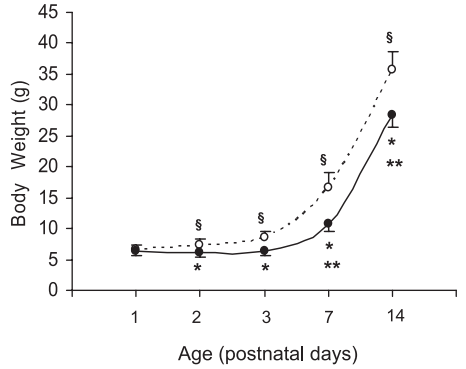

Figure 1. Effects of DEX on body weight. DEX administration (*closed symbols*) induces significant growth retardation compared with control group (*open symbols*). n = 12–35 per group. Values are means ± SD. *$p < 0.05$ DEX *vs* age-matched controls. **$p < 0.05$ within DEX-treated group compared with d 1. §$p < 0.05$ within SAL treated group compared with d 1.

Acute and chronic effects of neonatal DEX treatment on plasma insulin and glucose levels. Hyperglycemia was found at d 2 in rats treated with DEX, however, blood glucose levels decreased to the control level at 7 d and a significantly lower blood glucose level was found at 14 d of age (Table 3). Compared with the SAL-treated rats, DEX-treated rats showed significantly decreased plasma insulin levels at 2 d of age. Elevated plasma insulin was found at 7-d-old rat pups after DEX treatment, which, combined with normal blood glucose level, indicates an insulin resistant state. No differences in plasma insulin levels were found between SAL- and DEX-treated groups at 14 d of age.

Acute and chronic effects of neonatal DEX treatment on blood lipid profile and ketone bodies concentrations. DEX treatment led to a sustained increase in total plasma cholesterol concentrations (Table 3). Separation of plasma lipoproteins by FPLC revealed that increased cholesterol at 2 d of age was mainly confined to LDL- and HDL-sized fractions (Fig. 2*A*). In contrast, at 7 and 14 d, cholesterol was more abundantly present in VLDL and LDL-sized fractions (Fig. 2, *C* and *E*). Plasma triglyceride concentrations were low in 2-d-old DEX-treated rats (Table 2), mainly due to reduction in VLDL-sized fractions (Fig. 2*B*). Significantly elevated plasma triglyceride concentrations were observed at 7 d after DEX treatment, with particularly increased amounts of triglycerides in VLDL-sized fractions (Fig. 2*D*). No difference was found in plasma triglyceride concentration at 14 d between SAL- and DEX-treated groups (Table 2). At this point, plasma triglycerides were still predominantly associated with VLDL and less with LDL- and HDL-sized fractions in DEX-treated rats compared with controls (Fig. 2*F*). Plasma concentrations of nonesterified fatty acid (NEFA) and ketone bodies were increased in DEX-treated group at 2 d of age, yet, no significant differences existed at 7 and 14 d between both groups.

Table 2. *Effects of DEX on heart and liver weight, heart weight/body weight and liver/body weight ratios at different postnatal ages*

	Day 2		Day 7		Day 14	
	SAL	DEX	SAL	DEX	SAL	DEX
Heart weight (mg)	55.1 ± 5.4	40.5 ± 2.7*	122.9 ± 14.1	93.5 ± 10.9*	205.2 ± 13.3	172.8 ± 15.0*
Liver weight (mg)	299.7 ± 32.6	313.9 ± 25.8	548.3 ± 91.0	376.6 ± 51.0*	1022.4 ± 108.8	813.0 ± 91.0*
Heart weight/body weight (mg/g)	7.12 ± 0.75	7.45 ± 0.71	7.47 ± 0.72	8.65 ± 1.02*	5.79 ± 0.53	6.13 ± 0.60
Liver weight/body weight (mg/g)	38.53 ± 1.98	57.48 ± 2.69*	32.99 ± 1.31	34.73 ± 3.86	28.72 ± 1.92	28.73 ± 2.36

Values are expressed as means ± SD. (n = 11–12 per treatment group per age).
* $p < 0.05$ DEX *vs* age-matched control.

Table 3. *Effects of DEX on concentrations of blood glucose, plasma insulin, triglyceride, cholesterol, NEFA and ketone bodies at different postnatal ages*

	Day 2		Day 7		Day 14	
	SAL	DEX	SAL	DEX	SAL	DEX
Plasma insulin (pM)	298 ± 146	175 ± 63	175 ± 71	342 ± 153*	50 ± 18	51 ± 23
Blood glucose (mM)	6.45 ± 0.88	12.04 ± 2.87*	9.00 ± 1.33	7.64 ± 1.64	6.74 ± 1.33	5.58 ± 0.89*
Plasma cholesterol (mM)	2.27 ± 0.20	3.23 ± 0.25*	4.25 ± 0.28	6.55 ± 0.60*	4.14 ± 0.45	5.03 ± 0.33*
Plasma triglyceride (mM)	0.82 ± 0.18	0.56 ± 0.08*	1.56 ± 0.60	3.95 ± 1.16*	1.82 ± 0.30	2.33 ± 0.98
Plasma NEFA (mM)	1.09 ± 0.13	1.49 ± 0.07*	1.68 ± 0.17	1.98 ± 0.36	2.26 ± 0.33	2.13 ± 0.39
Plasma acetoacetic acid (mM)	0.42 ± 0.06	1.19 ± 0.32*	0.11 ± 0.04	0.32 ± 0.12*	0.29 ± 0.05	0.31 ± 0.07
Plasma β-hydroxybutyric acid (mM)	1.43 ± 0.02	2.42 ± 0.38*	0.50 ± 0.14	0.67 ± 0.12	0.96 ± 0.21	0.74 ± 0.24

Values are the means ± SD (n = 6–12 per treatment group per age).
* $p < 0.05$ DEX *vs* age-matched SAL-treated control.

Effects of DEX treatment on hepatic fatty acid and glucose metabolism. Hepatic triglyceride content was significantly decreased at 2- and 7-d-old DEX-treated animals, whereas triglyceride accumulation (steatosis) was clearly present at 14 d (Fig. 3). ORO staining revealed uniformly distributed fat droplets in liver of DEX-treated rats (Fig. 4, *A* and *B*). PAS staining revealed that DEX treatment led to hepatic glycogen accumulation at d 2 (not shown) and reduced amounts at 14 d of age (Fig. 4, *C* and *D*).

DEX led to increased hepatic mRNA levels of genes involved in fatty acid uptake and control of fatty acid oxidation such as PPARα and carnitine palmitoyltransferase 1A (CPT1A) at 2 d of age, suggesting enhanced hepatic fatty acid oxidation (Fig. 5). In contrast, at 7 d, some of these genes were down-regulated, which may be due to a suppressive effect of elevated plasma insulin at that age. Decreased expression of lipogenic genes such as fatty acid synthase (FAS) was noted at 2 d, and sterol regulatory element binding protein 1c (SREBP1c) at 2 and 7 d with DEX treatment. All these alterations were transient and normalized at 14 d of age.

Compared with SAL, DEX increased expression of hepatic gluconeogenic genes at 2 d of age, as characterized by up-regulation of PEPCK and G6PHT expression. Increased expression of some other important genes such as glycogen synthase (GS), glucokinase (GK), and glycogen phosphorylase (GP), together with up-regulated G6PHT, at the mRNA level suggests increased rates of hepatic glucose and glycogen cycling at that age. However, although hepatic PEPCK mRNA continued to be higher in 7-d-old DEX-treated rats, decreased

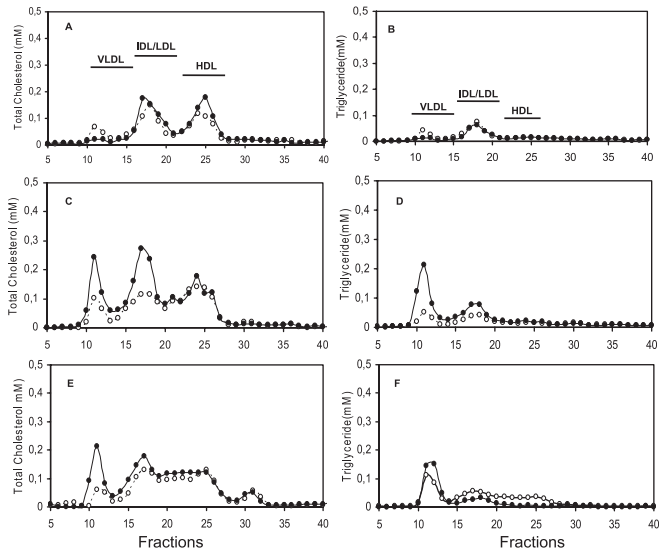

Figure 2. Fast protein liquid chromatography separation of plasma cholesterol (*A, C, E*) and triglyceride (*B, D, F*) from the SAL- (*open symbols*) and DEX-treated (*closed symbols*) rats at different ages. Plasma samples were pooled (n = 10) at each age. *A* and *B*: 2 d; *C* and *D*: 7 d; *E* and *F*: 14 d of age.

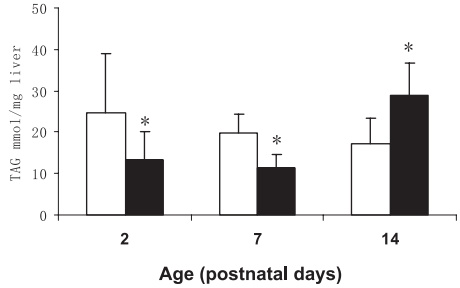

Figure 3. Effects of DEX on hepatic triglyceride content. Compared with controls, DEX administration results in significantly reduced triglyceride in 2 and 7 d, but an increased triglyceride at 14 d. n = 10 per group. Values are the means ± SD. SAL-treated group (*open bars*), DEX -treated group (*closed bars*). *$p < 0.05$ DEX *vs* age-matched control.

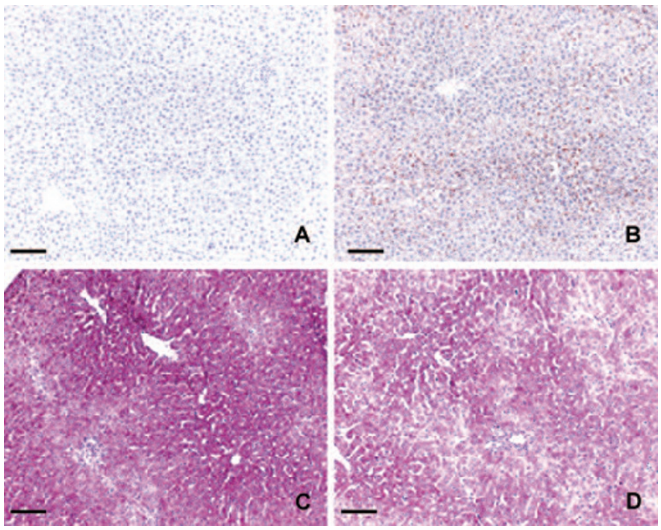

Figure 4. PAS staining shows reduced hepatic glycogen at 14 d with DEX treatment compared with age-matched control. $n = 6$ per group. *A:* SAL; *B:* DEX. ORO staining shows accumulated neutral fat in liver sections from DEX-treated rats at d 14 compared with control. $n = 4$ per group. *C:* control; *D:* DEX. Scale bars: 20 μm.

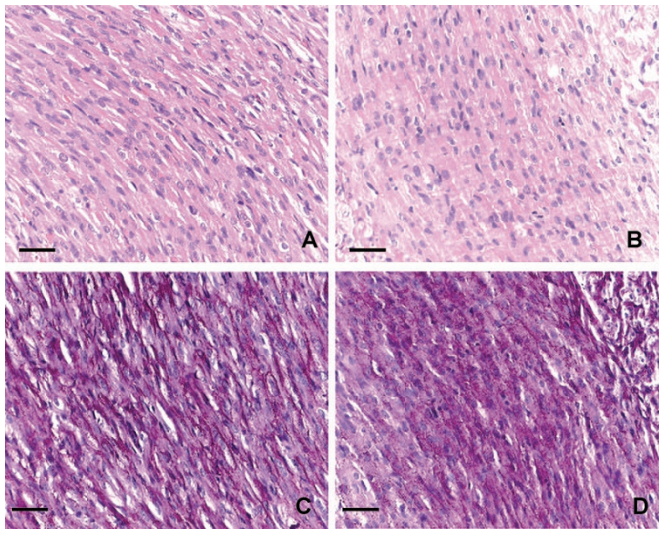

Figure 6. H&E and PAS staining of heart sections from 7-d-old rats. H&E staining showed hypertrophy in DEX-treated rats. *A:* SAL; *B:* DEX. There were no obvious differences found in PAS staining between two groups. *C:* SAL; *D:* DEX. $n = 6$ per group. Scale bars: 10 μm.

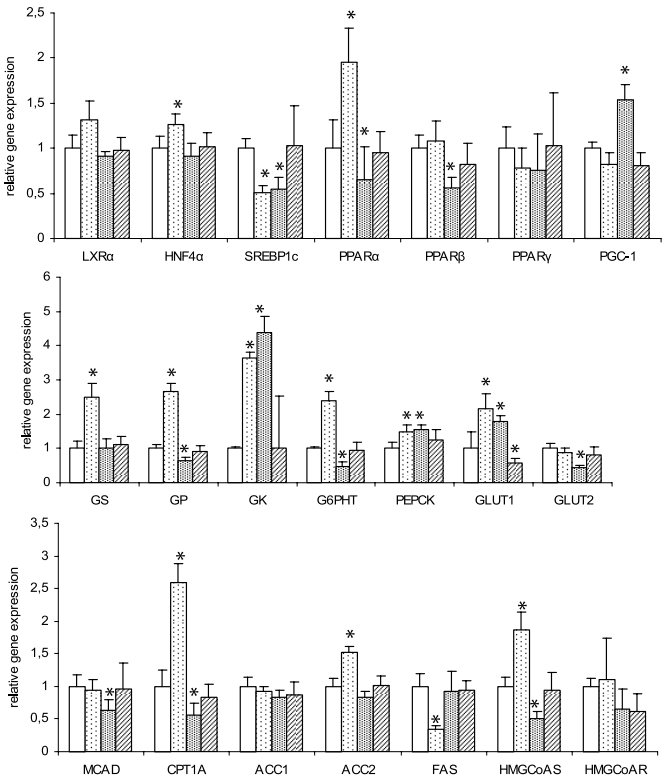

Figure 5. Effects of DEX on hepatic gene expression involved in fatty acid and glucose metabolism at different ages compared with SAL: SAL group (□) is set at 1, 2 d DEX (▨), 7 d DEX (▩), and 14 d DEX(▨). *$p < 0.05$ DEX *vs* age-matched control. $n = 7$ per group. *ACC1 and 2,* acetyl-CoA carboxylase 1 and 2; *FAS,* fatty acid synthase; *GK,* glucokinase; *GLUT1 and GLUT2,* glucose transporter 1 and 2; *GP,* glycogen phosphorylase; *GS,* glycogen synthase; *HMGCoAR,* hydroxymethylglutaryl-CoA reductase; *HMGCoAS,* hydroxymethylglutaryl-CoA synthase, mitochondrial; *HNF4α,* Hepatic nuclear factor 4α; *LXRα,* liver X receptor alpha; *MCAD,* medium chain acyl-CoA dehydrogenase; *PGC-1,* peroxisome proliferator-activated receptor gamma, co-activator 1; *PPARα, [gerds], βγ,* peroxisome proliferator-activated receptor alpha, beta, gamma.

G6PHT and glycogen phosphorylase (GP) suggest the decease in glycogen degradation. None of these genes was found to be differently expressed in the liver of 14-d-old rats of both groups (Fig. 5).

Effects of neonatal DEX treatment on expression of cardiac genes involved in energy metabolism. Confirming a previous study (21), H&E staining indicated the presence of enlarged cardiomyocyte in 7-d-old rat pups treated with DEX compared with controls (Fig. 6, *A* and *B*), without differences in PAS (Fig. 6, *C* and *D*) or ORO staining (data not shown).

Cardiac expression of genes involved in control of fatty acid and lipoprotein uptake and metabolism such as PPARα, PPAR[gerds], lipoprotein lipase (LPL), CD36, CPT1A, CPT1B, very LDL receptor (VLDLR), LDL receptor (LDLR), long-chain acyl-CoA dehydrogenase (LCAD), medium chain acyl-CoA dehydrogenase (MCAD), uncoupling protein (UCP) 2 and 3 was found to be significantly increased in hearts of 2-d-old DEX-treated rats, which indicates acute stimulatory effects of DEX on cardiac fatty acid utilization (Fig. 7). Expression of most of these genes, however, was reduced to control levels at d 7. DEX enhanced cardiac expression of genes involved in glucose transport and glycolysis such as glucose transporter 1 and 4 (GLUT1 and 4), and hexokinase 2 (HK2) at 2 d of age. Particularly cardiac pyruvate dehydrogenase kinase isoenzyme 4 (PDK4) mRNA was increased by DEX. These effects were transient and no significant differences between the groups were seen at 14 d. Increased cardiac gene expression of insulin receptor (IR) at mRNA level was only observed in 2-d-old rats upon DEX treatment.

Postnatal DEX treatment led to an increase in cardiac atrial natriuretic peptide (ANF), desmin, and troponin T (TnnT2) mRNA content at 2 d of age. Thereafter, cardiac ANF and TnnT2 mRNA levels returned to control level at 14 d of age, whereas desmin mRNA content was slightly decreased in DEX-treated rats at that time point.

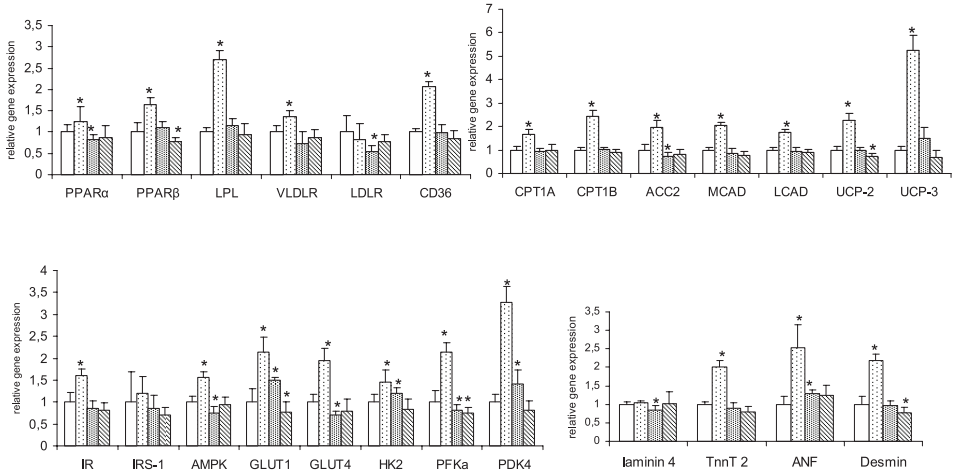

Figure 7. Effects of DEX on cardiac gene expression involved in fatty acid, glucose metabolism, and structure at different ages compared with SAL. SAL group (□) is set at 1, 2 d DEX (▨), 7 d DEX (▩) and 14 d DEX (▧). *$p < 0.05$ DEX *vs* age-matched SAL-treated control. $n = 6–12$ per group. *AMPK,* AMP-activated protein kinase; *CD36,* CD36 antigen; *CPT1A and B,* carnitine palmitoyltransferase A and B; *GLUT4,* glucose transporter 4; *HK2,* hexokinase2; *IR,* insulin receptor; *IRS-1,* insulin receptor substrate 1; *LCAD,* long-chain acyl-CoA dehydrogenase; *LDLR,* LDL receptor; *LPL,* lipoprotein lipase; *PDK4,* pyruvate dehydrogenase kinase isoenzyme 4; *PFKa,* phosphofructokinase, muscle, A-type; *VLDLR,* very LDL receptor.

DISCUSSION

Treatment of rat pups postnatally with DEX resulted in acute changes in energy metabolism, both with respect to substrate availability and hepatic and cardiac gene expression profiles. In a substantial number of cases, these changes were transient, *i.e.* no differences were observed between DEX- and SAL-treated groups at the age of 14 d. On the other hand, there were effects of early overexposure to DEX that persisted until 14 d of age, particularly at the level of plasma and hepatic metabolite concentrations. As a consequence, early exposure to DEX resulted in marked alterations in plasma lipoprotein composition at 14 d of age and a clear hepatic steatosis.

As reported previously (22), early exposure to DEX resulted in impaired growth: no catch-up growth occurred during the initial 14 d of life. In fact, it has been reported that neonatal exposure to DEX leads to sustained reduction in body weight gain which might be due to suppression of growth hormones and the IGF axis (23,24). In the present study, both liver and heart weight were reduced in DEX-treated animals, however, upon normalization to body weight, no differences between DEX- and SAL-treated rats were observed except an increased heart/body weight ratio at 7 d of age. Microscopic examination indicated the presence of cardiac hypertrophy at this time point. There were no apparent signs of cardiac glycogen or fat accumulation observed upon PAS or ORO staining at any age between SAL- and DEX- treated animals.

DEX treatment acutely led to an increase in plasma nonesterified fatty acid concentrations that might be attributable to enhanced lipolysis caused by lower plasma insulin levels. The latter may be a consequence of acute inhibitory effects of DEX on insulin secretion (25). Lower plasma insulin levels, in turn, might reduce glucose uptake by peripheral tissue. Yet, consistent with previous studies (26), DEX administration induced hepatic gluconeogenesis by up-regulating PEPCK and G6PHT gene expression at 2 d of age. Reduced glucose uptake by peripheral tissue and increased hepatic gluconeogenesis likely contribute to hyperglycemia at this time point. Interestingly, the cycling between glucose and glucose-6-phosphate, glucose-1-phosphate, and glycogen appeared to be increased as deduced from gene expression profiles, confirming a study conducted by Wajngot *et al.* (27) in healthy human subjects treated with DEX. This might reflect a regulatory mechanism to limit net hepatic glucose production. In addition, decreased fatty acid synthesis and increased fatty acid oxidation induced by DEX treatment, suggested by the expression level of responsible genes, might contribute to lower hepatic triglyceride content at this age. As reported previously (13), DEX led to an insulin resistant state at 7 d of age, as characterized by increased plasma insulin concentration and sustained increased hepatic gene expression of PEPCK, although blood glucose level was still kept at normal levels. Hyperinsulinemia might be caused by increased insulin secretion, which has also been found by Nicod *et al.* (28) in human studies. The molecular mechanism behind these observations is still unclear. Hypertriglyceridemia is frequently encountered in patients with insulin resistance. Data suggest that it is due to an inability of insulin to suppress hepatic VLDL secretion (29). There were no differences in hepatic "lipid" gene expression between SAL- and DEX-treated animals at 14 d old, indicating no persistent alteration in hepatic gene expression to occur in this experimental setting. However, increased VLDL-associated cholesterol and triglycerides, *i.e.* an atherogenic lipid profile (30), persistent until this age. Significantly reduced blood glucose concentration in DEX-treated animals at this age might be due to increased peripheral glucose uptake, yet, this remains to be established experimentally. Consistent with a previous study (31), hepatic steatosis was observed in 14-d-old animals upon DEX treatment. Whether this condition will persist until adult life and contributes to impaired hepatic insulin sensitivity remains to be determined.

The heart is a preferentially fatty acid-utilizing organ. An impressive amount of data suggest that inappropriately increased fatty acid utilization might be critical for cardiomyopathy in diabetes due to increased oxidative stress and accumulation of toxic fatty acid metabolites (11). We found that DEX administration led to increased cardiac fatty acid utilization at 2 d of age, as deduced from the elevated expression of genes involved in cardiac fatty acid uptake, transport, and oxidation. Elevated plasma fatty acid and triglyceride levels, especially the acute elevation of FFA concentrations, might be responsible for increased cardiac fatty acid metabolism, since it has been demonstrated that fatty acids up-regulate genes

controlling cardiac fatty acid transport and oxidation through activation of PPARα (32). Significantly increased cardiac pyruvate dehydrogenase kinase isoenzyme 4 gene expression might be induced directly by DEX, as observed in Morris hepatoma cells (33), leading to simultaneous suppression of cardiac glucose oxidation. Thus, increased cardiac blood glucose supply can only be metabolized by glycolysis since glucose oxidation is largely inhibited. At 7 d of age, expression most of these genes was already back to control levels. However, transient cardiac hypertrophy is prominent at this time point. Transient cardiac hypertrophy has been described in clinical trials in newborns upon GC treatment (3,34) without delineation of a clear mechanisms. In the present study, we found that some genes involved in cardiac hypertrophy, such as ANF, desmin (35), and TnnT2 (36), were already upregulated at 2 d of age, *i.e.* before the occurrence of an increased heart/body weight ratio. In addition, it has been revealed that enhanced glycolysis in cardiomyocytes may elevate endothelin-1 expression (37), which is implicated in pathologic cardiac hypertrophy (38). Therefore, we assume that increased cardiac glycolysis and/or increased expression of cardiac cytoskeleton genes might be involved in the pathogenesis of transient cardiac hypertrophy caused by DEX administration. Only reduced gene expression of desmin persisted at 14 d of age, which may predispose to impaired cardiac function in later life, such as cardiac dilatation and heart failure (39).

In conclusion, neonatal DEX treatment has transient effects on hepatic and cardiac energy metabolism and sustained effects on plasma triglyceride and lipoprotein profiles in suckling rats. Whether these alterations predispose to hepatic abnormalities of lipid metabolism and cardiovascular disease in later life needs to be further evaluated. Although it is obviously not correct to extrapolate data from this animal experiment to human situations, data support the notion that the continued prescription of postnatal steroids should be carefully considered.

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Table 1. *Primer sequences*

Gene name	Forward primer	Reversed primer	TaqMan probe 5′–3′
CD36	GAT CGG AAC TGT GGG CTC AT	GGT TCC TTC TTC AAG GAC AAC TTC	AGA ATG CCT CCA AAC ACA GCC AGG AC
CPT1a	CAG TGG GAG CGA CTC TTC AAT	GCC CTC TGT GGT ACA CAA CAA	CCT GGG GAA GAG ACA GAC ACC ATC CAA C
G6PHT	TGG CTG GGT TAC CCT TCA GTA	GAT CAC TTC TGC CAC CCA GAA	TCG CCA AGC ACT ATA GCT GGA GCA CA
GK	CCT GGG CTT CAC CTT CTC CTT	GAG GCC TTG AAG CCC TTG GT	CAC GAA GAC CTA GAC AAG GGC ATC CTC CTC
GLUT-1	TGT GCA GCA GCC TGT GTA TG	CCA GCC AGA CCA ATG AGA TG	CCT TCA CTG TGG TGT CGC TGT TCG TC
GLUT-2	GCA TCA GCC AGC CTG TGT ATG	GCA GCA CAG AGA CAG CTG TGA	CCA TCG GCG TTG GTG CCA TCA AC
GP	AAG GAG CTG AGG CTG AAG CA	GCC TTG AAA CGT CGG ATG AC	CTG CAG GGT CGC AGC CAC CAC
GS	CGC ACG TGT TTA CCA CAG TGT	CAG GCT TCC TCT TCA GCA TGT	TCC GCC TCG ATG GCT GTG ATT TCT
HMGCoAR	CCG GCA ACA ACA AGA TCT GTG	ATG TAC AGG ATG GCG ATG CA	TGT CGC TGC TCA GCA CGT CCT CTT C
HMGCoAS mito	TGG TGG ATG GGA AGC TGT CTA	CTG CGG TAA GCT GCA TAG CAT	CCA AGG CCC GCA GGT AGC ACT G
HNF4-alpha	ATG CCA AGG GGC TGA GTG AC	GCC GGT CGT TGA TGT AAT CCT	CAC CTG TGA CCG CAG CCG CTT G
IR	TGA GTC AGC CAG TCT TCG AGA A	GCC ATC AGT TCC ATC ACT ACC A	CCA CGT GAT GAC AGG TGA AGC CCT TC
IRS-1	AGC ACC TGG TGG CTC TCT ACA	CAG CTG CAG AAG AGC CTG GTA	CTC GCT ATC CGC CGC AAT GGC
LCAD	TAC GGC ACA AAA GAA CAG ATC G	CAG GCT CTG TCA TGG CTA TGG	CAC TTG CCC GCC GTC ATC TGG
MCAD	CGG AAG TTT GCC AGA GAG GAA	TCC CAG GCT CTC TTG ATG AGA	TCG CCC CAG ACT ACG ATA AAA GCG GG
PFKa	CCA AAG ACG TGA CCA AGG CTA T	GCT TGT ATA CCT CCC AGT TGT TCA T	CCG GCC TCT CAG CTT AAT GGC TTC A
PGC-1	GAC CCC AGA GTC ACC AAA TGA	GGC CTG CAG TTC CAG AGA GT	CCC CAT TTG AGA ACA AGA CTA TTG AGC GAA CC
UCP-2	CGA AGC CTA CAA GAC CAT TGC	CAC CAG CTC AGT ACA GTT GAC	CAG AGG CCC CGG ATC CCT TCC
UCP-3	CCA TGA TAC GCC TGG GAA CTG	TGT TGG GCC AAG TCC CTT TC	CGA TGG TTC TGT AGG CAT CCA TAG TCC CTC
AMPK	CTC TGG GCA TCT TTG TAC AGC A	CAC CAC ACG CCC TTT CTC A	CAC CAC AGG CAA GGC GGA GAC TC
ANP	AGG CCA TAT TGG AGC AAA TCC	AGG TGG TCT AGC AGG TTC TTG AAA	CAT CAG ATC TGT GTT GGA CAC CGC ACT GT
Desmin	TGG TAC AAG TCC AAG GTT TCA GAC T	CTG GTG TCG GTA TTC CAT CAT CT	AAG AAC AAC GAT GCG CTG CGC C
TnnT2	GAG AGT GGA CTT TGA TGA CAT CCA	CGA AGT GAG CCT CGA TCA GAG	TCG TTC AGG TCC TTC TCC ATG CGC
Laminine	GCG GGA CCA TGA GAA ACA AC	TCA TCA AGT TCC TCA AGA GTG AGC	CCA ACG GCC TCC ATC TGT TCC TTC