Original Article

MOLECULAR METABOLISM

Dwarfism and insulin resistance in male offspring caused by α1-adrenergic antagonism during pregnancy

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ABSTRACT

Objective: Maternal and environmental factors control the epigenetic fetal programming of the embryo, thereby defining the susceptibility for metabolic or endocrine disorders in the offspring. Pharmacological interventions required as a consequence of gestational problems, e.g. hypertension, can potentially interfere with correct fetal programming. As epigenetic alterations are usually only revealed later in life and not detected in studies focusing on early perinatal outcomes, little is known about the long-term epigenetic effects of gestational drug treatments. We sought to test the consequences of maternal α1-adrenergic antagonism during pregnancy, which can occur e.g. during hypertension treatment, for the endocrine and metabolic phenotype of the offspring.

Methods: We treated C57BL/6NCrl female mice with the α1-adrenergic antagonist prazosin during pregnancy and analyzed the male and female offspring for endocrine and metabolic abnormalities.

Results: Our data revealed that maternal α1-adrenergic blockade caused dwarfism, elevated body temperature, and insulin resistance in male offspring, accompanied by reduced IGF-1 serum concentrations as the result of reduced hepatic growth hormone receptor (*Ghr*) expression. We subsequently identified increased CpG DNA methylation at the transcriptional start site of the alternative *Ghr* promotor caused by the maternal treatment, which showed a strong inverse correlation to hepatic *Ghr* expression.

Conclusions: Our results demonstrate that maternal α1-adrenergic blockade can constitute an epigenetic cause for dwarfism and insulin resistance. The findings are of immediate clinical relevance as combined α/β-adrenergic blockers are first-line treatment of maternal hypertension.

Keywords Brown fat; Pregnancy; Thermogenesis; Fever; Insulin resistance; IGF-1

1. INTRODUCTION

Environmental and lifestyle factors such as diet, obesity, and physical activity can influence epigenetic processes by modulating DNA methylation, histone acetylation, and miRNA expression [1–3]. Thereby, the environment can adjust gene transcription rates, leading to changes in cellular physiology [3,4] and gene transcription [5,6] that can persist throughout the entire life-span, subsequently affecting the adult phenotype. Thus, the investigation of *in utero* programming is an important approach to reveal mechanisms underlying the development of metabolic disorders without genomic inheritance [7,8], which is of particular relevance as genome-wide association studies were largely disappointing in identifying major genetic causes for metabolic disorders [9].

The term "fetal programming" summarizes epigenetic changes that occur in the intrauterine environment by e.g. maternal hormonal, nutritional status, or stressful events during pregnancy [10,11]. Although maternal programming is thought to have beneficial effects on short-term offspring survival rate, several recent studies have shown that intrauterine modifications of the fetus' epigenetic profile are almost always associated with an increased risk to develop metabolic diseases during later life [3,4,10,11]. Maternal hormones such as glucocorticoids or thyroid hormones are especially important for *in utero* programming as they directly convey the endocrine situation of the mother to developing feto-placental tissues (reviewed in [12]). Similarly, maternal malnutrition as well as overnutrition can cause deleterious changes in the epigenome of the offspring [4,8,13,14]: Famine during pregnancy causes intrauterine growth restriction (IUGR) and leads to metabolic

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Abbreviations: iBAT, interscapular brown adipose tissue; Cyclo, cyclophilin D; DNP, 2,4-dinitrophenol; GH, growth hormone; GHR, growth hormone receptor; HPRT, hypoxanthine-guanine phosphoribosyltransferase; IGF-1, insulin-like growth factor 1; IUGR, intrauterine growth restriction; iWAT, inguinal white adipose tissue; gWAT, gonadal white adipose tissue; UCP1, uncoupling protein 1

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diseases later in life [15,16], and maternal overnutrition and excessive gestational weight gain also have been associated with a higher predisposition in obesity [17,18].

Likewise, pharmacological interventions to treat gestational disorders such as hypertension and preeclampsia can potentially affect epigenetic fetal programming; however, little is known about the epigenetic consequences of these treatments, as they are usually not detected in clinical studies aiming at assessing the pre- or early postnatal development of the offspring. The situation is particularly challenging with regard to hypertension as the most common complication in pregnancy [19,20], since classical treatment options such as ACE inhibitors or angiotensin II receptor antagonists are not recommended. Current guidelines, therefore, suggest drugs targeting α/β-adrenergic receptors such as labetalol as first line treatment choices; however, these therapeutic options are also controversially discussed, as β-blockers have been associated with small gestational age, preterm birth, and perinatal mortality [21,22]. In contrast, compounds antagonizing α-adrenergic signaling have been considered relatively safe with regard to early perinatal outcome [19,23,24].

Here, using the α1-adrenergic specific antagonist prazosin, we tested the long-term consequences of maternal α1-blockade in pregnancy for the endocrine and metabolic phenotype of the adult offspring.

2. MATERIALS AND METHODS

2.1. Animal husbandry

Wildtype C57BL/6NCrl mice were purchased from Charles River and kept on a constant 12-hour light/12-hour dark cycle at 22 $\pm$ 1 °C with free access to food and water (standard (1324) or breeding diet (1314) from Altromin (Germany)). All procedures were approved by the MELUR Schleswig—Holstein, Germany. Female mice at 3 months of age were kept in paired groups and treated with either 50 μg/ml prazosin or 800 μg/ml 2,4-dinitrophenol (DNP) in drinking water, starting two days before mating until the end of pregnancy, with bottles changed every second day. Following a positive plug check, females were separated (embryonic day 0.5, E0.5). At the age of 4—5 months, diurnal rhythms of locomotor activity were assessed by running-wheel activity. Data were analyzed with Clocklab Analysis software (Actimetrics, IL, USA). Body length was determined by measuring the distance between nose and tail base.

2.2. Glucose and insulin tolerance tests

Animals were fasted for 6 h before intraperitoneal injection of glucose (2.0 g/kg body weight, N = 4—8 per group at the age of 4—5 months). Blood glucose concentrations were measured in blood drawn from the tail vein using a glucometer (AccuCheck, Aviva, Germany). For the insulin tolerance test, mice received an intraperitoneal injection of 0.5 IU insulin/kg body weight (Novo Rapid, Novo Nordisk, Denmark).

2.3. Infrared thermography

Infrared thermography was performed at different time points during pregnancy (baseline, E18.5) and pictures were taken from the inner ear, interscapular brown adipose tissue (iBAT) area and tail surface. Pictures of newborn offspring (P5) were taken 3 min after the litter had been removed from the mother. Adult animals were freely moving for iBAT pictures, whereas tail pictures were taken after animals were kept on a temperature-controlled platform for 15 min.

2.4. Western blot analysis

For protein isolation, iBAT tissue was snap-frozen, and protein concentrations were determined using a bicinchoninic acid assay (Sigma, Germany). For immunological detection of the uncoupling protein 1 (UCP1), 5 μg of protein were separated on 12% SDS polyacrylamide gels and transferred onto polyvinylidene membranes (Millipore, Germany). Membranes were probed with a rabbit anti-UCP1 polyclonal antibody (1:10,000 dilution, previously used in [25]) and rabbit anti-GAPDH polyclonal antibody (1:10,000 dilution, #2118, Cell Signaling Technology, USA) followed by peroxidase-conjugated secondary antibody (polyclonal goat anti-rabbit-IgG at 1:5000 dilution, #P0448, DAKO). Antigens were visualized using an ECL Plus western blotting detection system (Chemi Doc Touch, BioRad, USA). Band intensity was quantified using ImageJ.

For *ex vivo* lipolysis, glycerol release from tissue explants (iBAT, iWAT, gWAT) was measured using commercially available free glycerol reagent and glycerol standard (Sigma—Aldrich).

2.5. Quantitative real-time PCR (qPCR)

RNA isolation was performed on snap-frozen tissue using RNeasy Kits (QIAGEN, Germany). Subsequent cDNA synthesis was carried out using the Molecular Biology RevertAid Strand cDNA Kit (Thermo Fisher Scientific, Germany). Quantstudio Applied Biosystems (Thermo Fisher Scientific, Germany) and SYBR Green PCR Master Mix (Roche, Germany) were used for qPCR analysis. Gene expression levels were normalized with one or two housekeeping genes (*Hprt*/*Gapdh* for pituitary, *Hprt*/*Cyclo* for liver, *Hprt*/*Cyclo* for hypothalamus and *Hprt* for gWAT) and PCR efficiency was corrected by the calculation of standard curves.

2.6. DNA extraction and analysis of DNA Methylation by bisulfite-pyrosequencing

Cytosine-methylation was quantified using bisulfite-pyrosequencing. Genomic DNA was extracted from snap-frozen liver or gWAT using the QIAamp DNA Mini Kit (Qiagen, Germany) and quantified using the Quantus Fluorometer (Promega, Germany). Genomic DNA (1650-2000 ng in 20 μl) was treated with sodium-bisulfite using the EpiTect Fast DNA Bisulfite Kit (Qiagen, Germany) and subsequent PCR was performed using the PyroMark PCR Kit (Qiagen, Germany). PCR and sequencing primers were generated with the PyroMark Assay Design Software 2.0 (Qiagen, Germany) or purchased from Qiagen (see Table S1). 10 μl of PCR product was used for pyrosequencing analysis on the PyroMark Q48 Autoprep with Q48 Advanced CpG Reagents (Qiagen, Germany). Quality was controlled using a standard curve of 0%, 50% and 100% methylated DNA and by testing for possible primer-self-annealing. Genome build GRCm38.5 was used for annotations.

2.7. IGF-1, C-peptide and total T4 ELISA

Serum IGF-1 (ab100695, Abcam, Germany), C-peptide (C-peptide 2, EZRMCP2-21K, Millipore, Merck Darmstadt, Germany), and total T4 (EIA 1781, DRG Instruments GmbH, Marburg, Germany) were determined by commercial ELISA kits.

2.8. Glycogen content in liver tissue

Glycogen content of snap-frozen liver tissue samples was determined as previously described [26].

2.9. Statistical analysis

GraphPad Prism 5 software was used to analyze the data. Values are represented as mean $\pm$ SEM. Statistical testing was performed using Student's t test with Welch's correction, one-way ANOVA with Bonferroni post-hoc-test or two-way repeated measurement ANOVA with Bonferroni post hoc test as indicated. $P < 0.05$ was considered

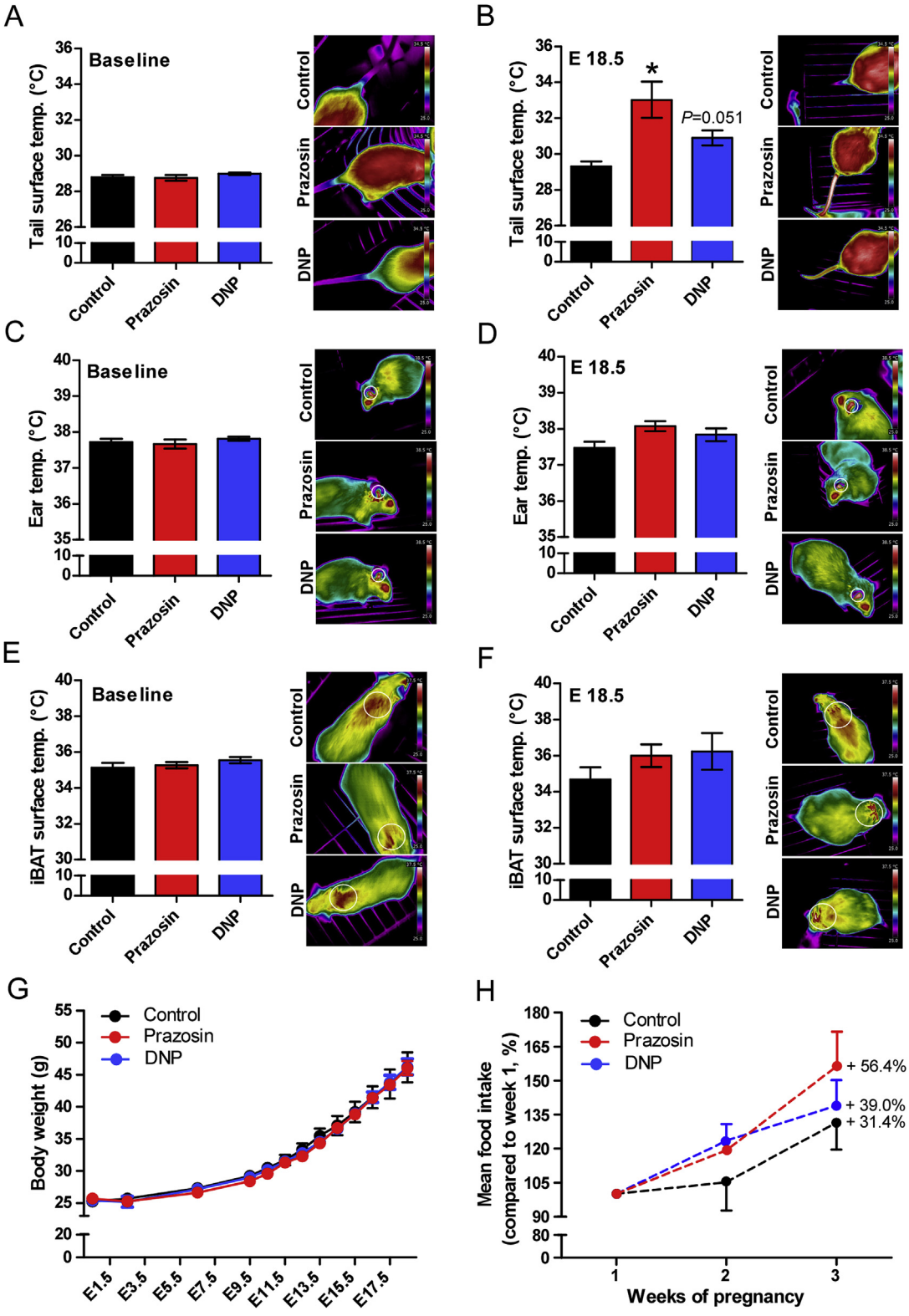

Figure 1: Prazosin and DNP treatment lead to increased heat loss over the tail surface of pregnant mice. Female mice were treated with either 50 μg/ml prazosin or 800 μg/ml DNP in drinking water from two days before mating until delivery. Heat dissipation over the tail surface of prazosin and DNP treated dams increased towards the end of pregnancy (A, B), but animals maintained their body temperature (C, D) by either dissipating excessive heat (DNP) or by activating endogenous heat production (prazosin). No significant changes in iBAT surface temperature could be detected (E, F). Litter size was similar (not shown) and the treatment had no effect on body weight gain (G), but the increase in caloric intake from gestation (week 1) until birth (week 3) was higher in prazosin treated animals (H; + 56.4%) and DNP treated animals (+39.0%) as compared to controls (+31.4%). N = 3–4 animals per group. All values are mean ± S.E.M. and $P < 0.05$ (*) with one-way ANOVA and Bonferroni post hoc test (A–F) or two-way repeated measurement ANOVA and Bonferroni post hoc test (G–H).

significant. The respective levels of significance and group sizes are stated in the figure legends.

3. RESULTS

3.1. Thermogenic and metabolic effects of DNP and prazosin treatment during pregnancy

To study the consequences of maternal α1-adrenergic blockade, we treated female mice during pregnancy with the specific α1-adrenergic antagonist prazosin. As the drug is known to induce an indirect activation of thermogenesis in mice through elevated heat loss over the tail surface [27], we treated a separate cohort of pregnant female mice with DNP, an artificial mitochondrial uncoupler that leads to an overall increase in thermogenesis and metabolism [28]. These mice were used as control to identify effects caused by the secondary elevation in maternal thermogenesis.

As expected, treatment of pregnant female mice with prazosin induced a significant elevation of heat dissipation over the tail surface (33.0 ± 1.0 °C, $P < 0.05$, Figure 1A,B) and a slight elevation of iBAT-temperature (36.0 ± 0.6 °C versus control: 34.7 ± 0.7 °C, Figure 1E,F), while maintaining body temperature (Figure 1C,D). DNP treatment provoked the production of heat and consequently DNP treated dams increased tail vasodilation (30.9 ± 0.4 °C versus control: 29.3 ± 0.3 °C, $P = 0.051$, Figure 1A,B) to avoid hyperthermia.

Since thermogenesis involves excess oxidation of lipids and glucose, animals have to adjust their daily caloric intake to avoid a negative energy balance. We therefore measured daily food intake and body weight throughout pregnancy to indirectly monitor energy balance and metabolism in prazosin and DNP treated dams. As expected, elevated thermogenesis was accompanied by a greater energy demand, which was reflected in a higher food intake of prazosin treated dams (+56.4%) and DNP treated dams (+39.0%) as compared to controls (+31.4%). Thereby animals treated with either drugs were able to keep up the positive energy balance required in pregnancy, and body weight gain resembled that of controls (Figure 1G,H).

3.2. Effects of DNP and prazosin treatment on offspring development

We subsequently investigated possible effects on metabolism and endocrine function in the offspring by monitoring body weight and endogenous heat production directly after birth. Male and female offspring of prazosin and DNP treated dams displayed normal birth weight and normal early postnatal development, indicating that no IUGR occurred (Figure 2A,D). From P6 on, however, offspring from prazosin and DNP treated mothers gained less body weight as compared to controls, with the exception of female pups from DNP treated dams ($P < 0.01$ and $P < 0.001$, Figure 2A,D). Interestingly, the changes in body weight were accompanied by a higher thermogenic capacity at P5 ($P < 0.05$ and $P < 0.001$, Figure 2B,E). While body weight alterations in the offspring from DNP treated dams were only transient and normalized after weaning, male offspring from prazosin treated females also had a significant leaner phenotype during adulthood ($P < 0.01$ at age P115, Figure 2C,F). These adult effects are likely not a consequence of elevated maternal thermogenesis, as they were absent in offspring of DNP treated mothers. Consequently, we focused our subsequent analysis on male offspring from prazosin treated dams (from here on referred to as prazosin offspring). Of note, the duration that the fathers were exposed to the prazosin drinking water during the mating process had no effect on the offspring phenotype (Figure S1A,B), thus likely excluding any paternal contribution to the phenotype.

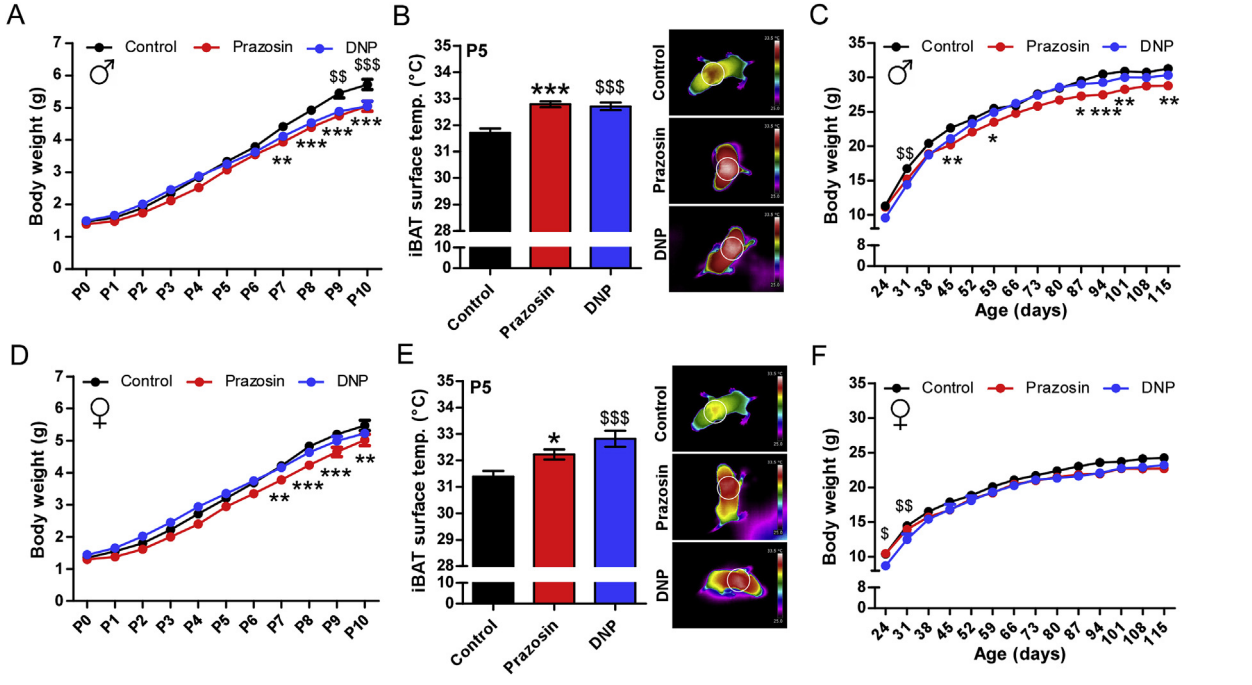

Figure 2: Offspring of prazosin and DNP treated dams display a reduction in body weight gain and a higher thermogenic capacity. Starting from the same body weight at birth, male offspring of prazosin and DNP dams and female offspring of prazosin dams gained less weight during the first days of postnatal development (P1-10) compared to controls (A and D); however, during adulthood, this effect persisted only in male offspring of prazosin dams (C and F). Infrared pictures of the back area were taken at P5, shortly before the emergence of fur, to quantify the thermogenic capacity of the offspring, which was significantly increased in offspring from all treatment groups (B and E). $N = 11-24$ males per group (A–C) or $N = 5-17$ females per group (D–F). All values are mean ± S.E.M. and $P < 0.05$ (* for prazosin, $ for DNP), $P < 0.01$ (** for prazosin, $$ for DNP) or $P < 0.001$ (*** for prazosin, $$$ for DNP) with one-way ANOVA and Bonferroni post hoc test (B and E) or two-way repeated measurement ANOVA and Bonferroni post hoc test (A, C, D and F).

3.3. Metabolic phenotype of prazosin offspring

To identify the mechanism underlying reduced body weight in male prazosin offspring, diurnal rhythms in locomotor activity were assessed by measuring voluntary running-wheel performance. Maternal treatment with prazosin did not affect daily patterns of activity or average light and dark phase activity levels (Figure 3A,B), implying that the lean phenotype of male offspring was not a result of neurotic or repetitive stereotyped behavior, which can alter energy expenditure and thereby body weight. Accordingly, skeletal muscle mass was similar to controls, except for M. soleus, which was significantly smaller in prazosin offspring ($P < 0.05$, Figure 3C). Absolute food intake was significantly reduced, likely contributing to the lower body weight ($P < 0.05$, Figure 3D); however, this was not caused by altered expression of hypothalamic genes governing energy intake such as neuropeptide Y (*Npy*), agouti-related peptide (*Agrp*), proopiomelanocortin (*Pomc*) or orexin (Figure S1C). Water intake was unchanged (Figure 3E).

Since prazosin offspring showed alterations in endogenous heat production during the first days after birth (Figure 2B), we tested if abnormal thermogenesis in the adult animal could contribute to the lower body weight. Measurements were performed using a rectal probe for body temperature recordings and infrared thermography for iBAT and tail surface temperature monitoring. Adult male prazosin offspring showed an elevation in body temperature ($P < 0.05$, Figure 4A), which was accompanied by an increase in iBAT surface temperature ($P < 0.01$, Figure 4B). As tail temperature was not elevated (Figure 4C), indicating an absent heat stress response, this points to a centrally induced elevation of body temperature (pyrexia). Indeed, when normalizing iBAT temperature to rectal body temperature, no difference was observed between control and prazosin offspring (Figure 4D). Even more importantly, when tail temperature was normalized to body temperature, it was significantly decreased, suggesting enforced tail vasoconstriction, which normally occurs

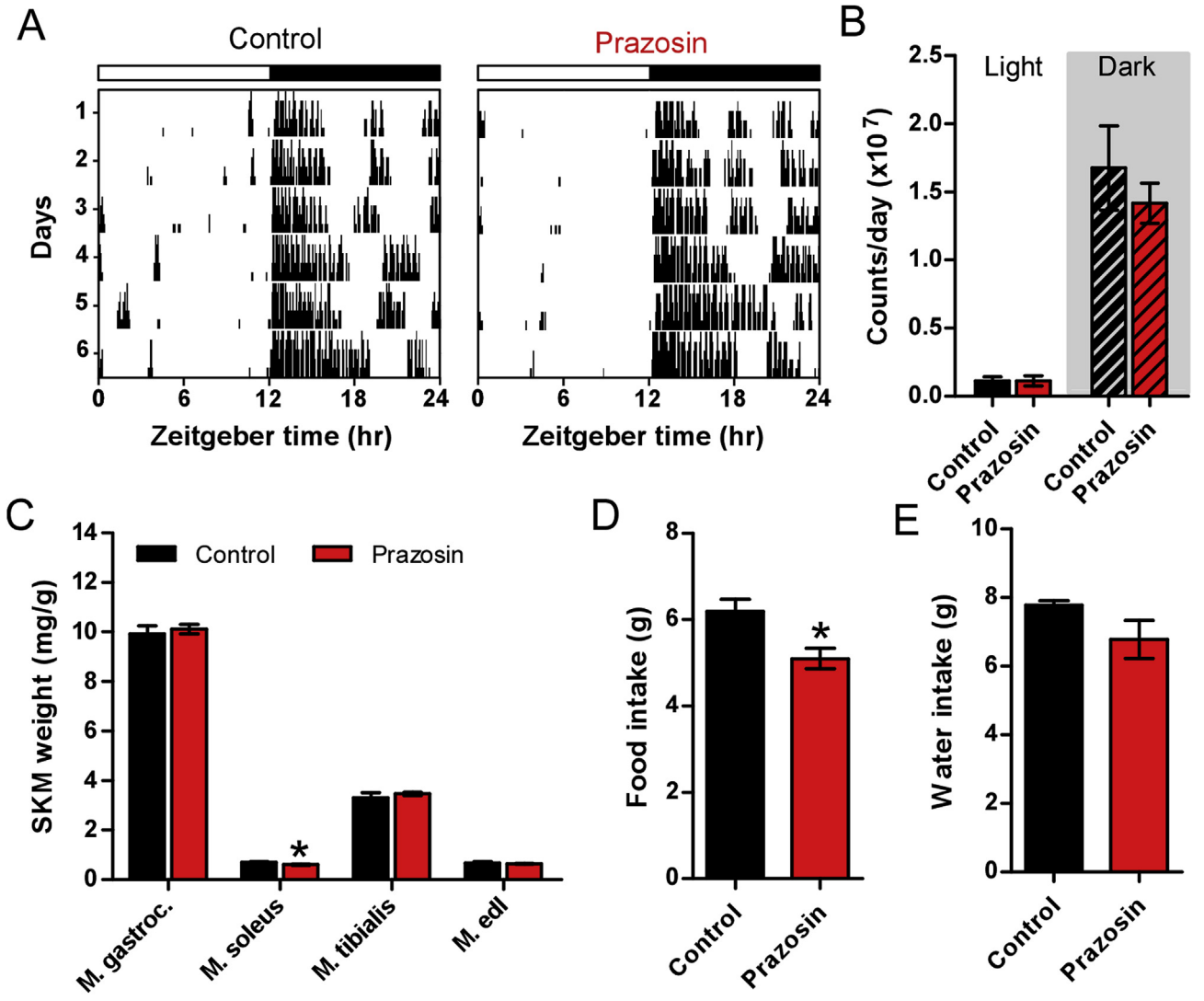

Figure 3: Maternal prazosin treatment has no influence on locomotor activity but reduces food intake in adult male offspring. Running wheel activity of male prazosin offspring was measured as an indicator of voluntary activity at the age of 4–5 months, black bars indicate the dark phase (A, one example per group). Neither daily nor nocturnal running wheel performance was affected by the maternal treatment (B), and only muscle mass of M. soleus was significantly changed (C). Food and water intake were measured in parallel and revealed a significant reduction in daily caloric intake in prazosin offspring (D, E). $N = 4–8$ animals per group (A, B, D, E) or $N = 8$ animals per group (C). All values are mean ± S.E.M. and $P < 0.05$ (*) with Student's t-test and Welch's correction. SKM: skeletal muscle.

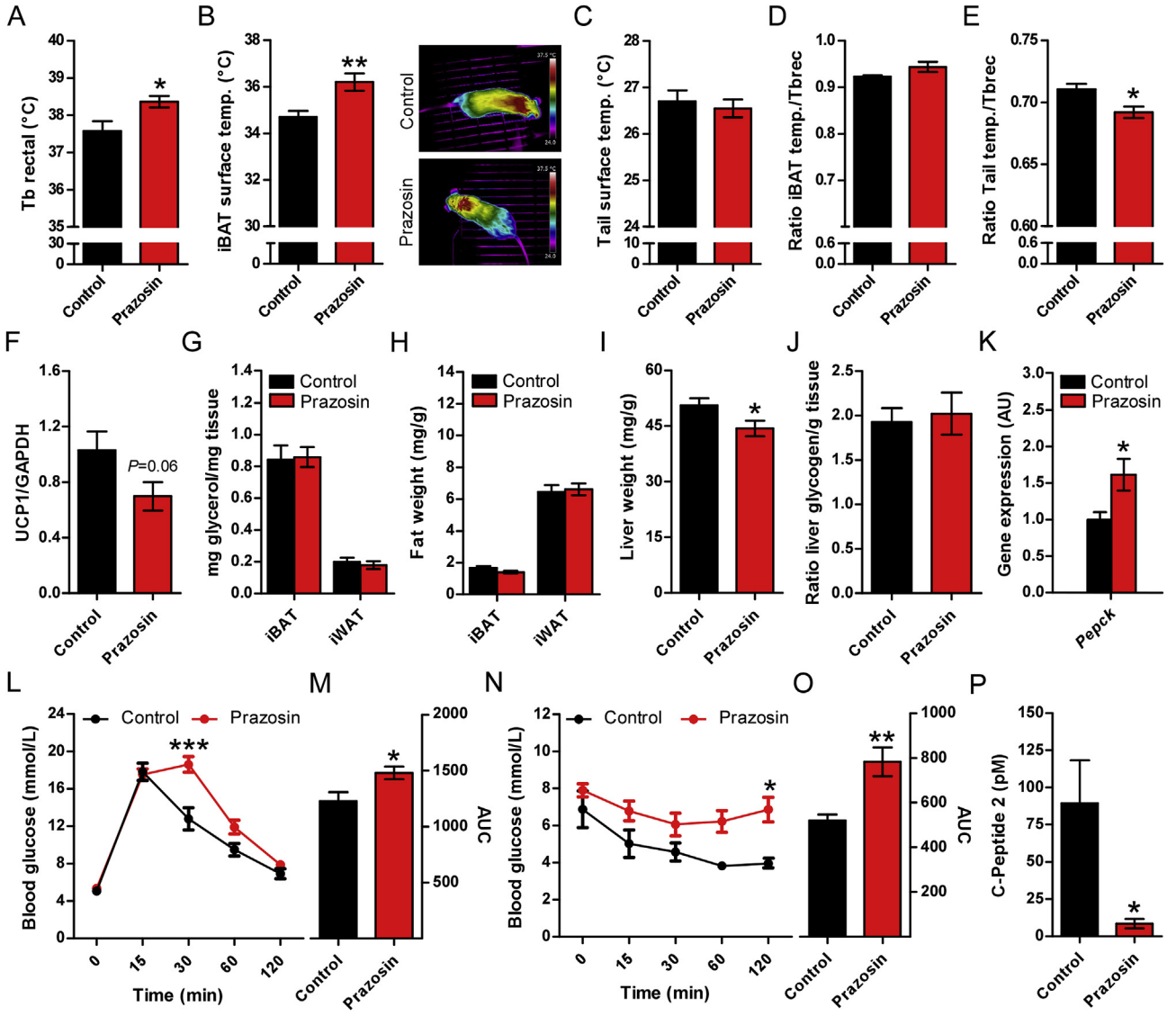

Figure 4: Metabolic changes caused by maternal prazosin treatment. Body temperature and iBAT surface temperature of male prazosin mice were significantly increased (A, B), while tail surface temperature remained unchanged (C). However, calculation of the ratio iBAT temperature/body temperature (D) and tail surface temperature/iBAT temperature (E) reversed observed effects. iBAT UCP1 protein levels tended to be lower in prazosin offspring (F) and further *ex vivo* analysis revealed no difference in basal lipolytic activity (G) or fat pad weight (H) of iWAT and iBAT (results for gonadal WAT are shown in Figure S1). Liver weight was decreased whereas hepatic glycogen stores were not altered (I, J), and expression levels of phosphoenolpyruvate-carboxykinase (*Pepck*) were increased (K). Offspring from prazosin dams showed an impaired glucose tolerance (L, M), and animals were suffering from an insulin resistance (N, O) in the presence of lower C-peptide serum concentrations (P). N = 4–8 animals per group (A–E, L–O) or N = 8 animals per group (F–K, P). All values are mean ± S.E.M. and $P < 0.05$ (*), $P < 0.01$ (**) or $P < 0.001$ (***) with Student's t-test and Welch's correction (A–K, M–P) or two-way repeated measurement ANOVA and Bonferroni post hoc test (L and N). AUC: area under the curve; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; Tbrec: rectal body temperature; UCP1: uncoupling protein 1.

during pyrexia ($P < 0.05$, Figure 4E). In line with these results, subsequent measurements of UCP1 protein (thermogenic protein of iBAT) expression levels showed no difference between prazosin offspring and controls (Figure 4F). Furthermore, no changes in fat tissue weight or *ex vivo* lipolytic activity of iBAT and inguinal white adipose tissue (iWAT) could be observed (Figure 4G,H and Figure S1D,E for gWAT), suggesting no additional recruitment of these tissues. Interestingly, liver wet weight was reduced in prazosin offspring ($P < 0.05$, Figure 4I), while hepatic glycogen levels were normal (Figure 4J) in the presence of elevated phosphoenolpyruvate-carboxykinase (*Pepck*) expression, a rate-limiting enzyme of hepatic gluconeogenesis (Figure 4K), and unaltered pyruvate kinase (*Pk*) expression, a marker for glycolysis (Figure S1E).

Although prazosin offspring displayed a leaner phenotype, which is often associated with improved metabolic function, we observed a reduced glucose clearance rate and a diminished insulin response ($P < 0.05$ (GTT, Figure 4L, M) and $P < 0.01$ (ITT, Figure 4N, O) for area under the curve), indicative of insulin resistance. This was accompanied by reduced serum C-peptide concentrations ($P < 0.05$, Figure 4P), suggesting that insulin release might also be impaired.

3.4. GH/IGF-1 signaling and dwarfism in prazosin offspring

Prompted by the significant reduction in body length of prazosin offspring ($P < 0.001$, Figure 5A), we hypothesized that a defect in the GH/IGF-1 axis could be the underlying cause of the phenotype. This was

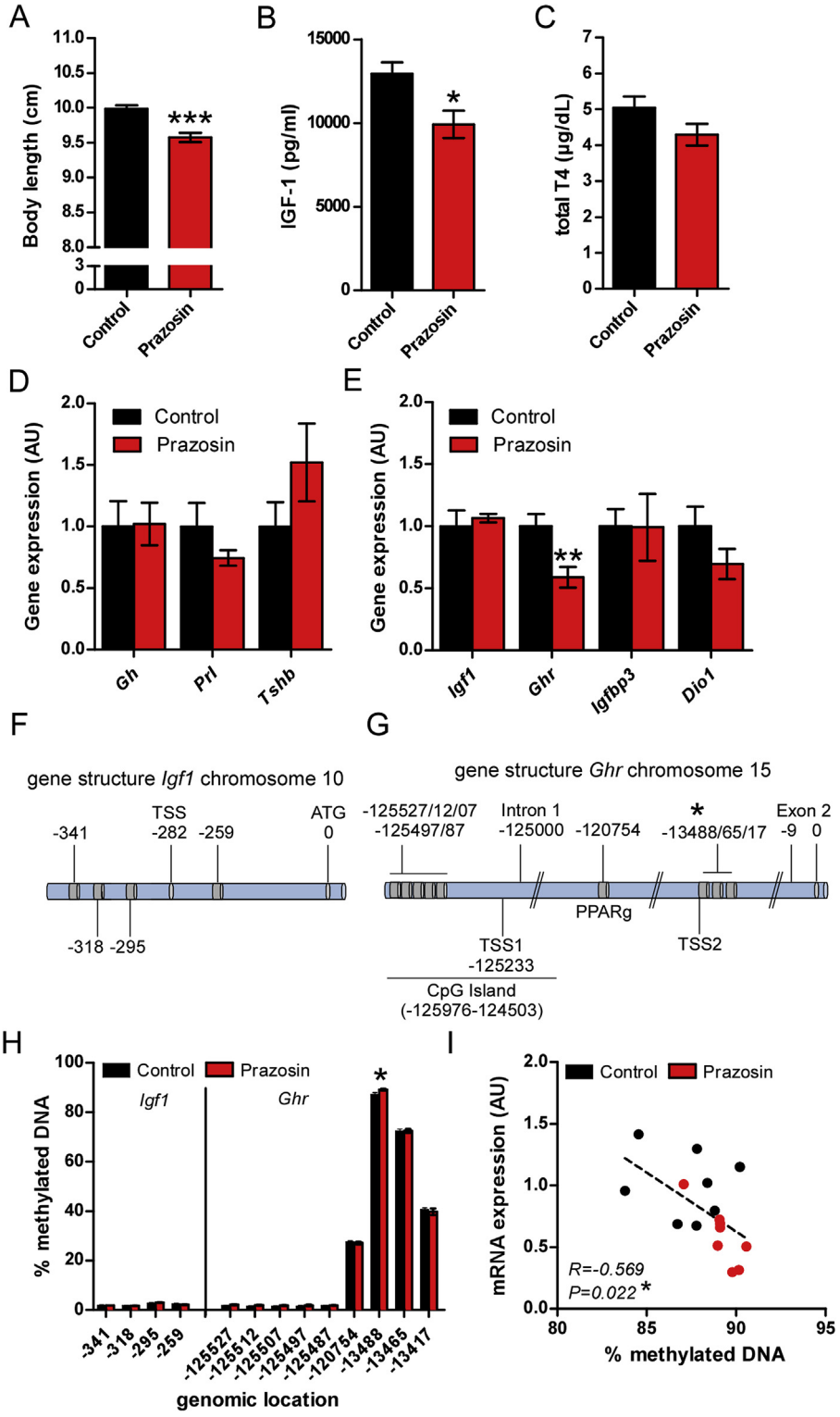

Figure 5: Male offspring from prazosin treated dams display characteristics of dwarfism. Body length was measured at the age of 4–5 months, and male prazosin offspring were significantly shorter when compared to age matched control animals (A). IGF-1 serum levels were decreased (B; control: N = 4, prazosin: N = 8), total T4 levels normal (C), and gene expression analysis of pituitary (D) and liver (E) mRNA revealed changes in growth hormone receptor (*Ghr*) expression. Hepatic DNA methylation of selected CpG sites in the *Igf1* (F) and *Ghr* (G) gene as assessed by bisulfite pyrosequencing (H). Hepatic *Ghr* mRNA expression and CpG -13488 DNA methylation for all animals was inversely correlated (I). N = 4–8 animals per group (B) and N = 8 animals per group (A, C–I). All values are mean ± S.E.M. and $P < 0.05$ (*), $P < 0.01$ (**) or $P < 0.001$ (***) with Student's t-test and Welch's correction (A–E) or Holm-Sidak correction for multiple testing (H) or Pearson's correlation (I). ATG: translational start; AU: arbitrary unit; *Dio1*: deiodinase 1; *Gh*: growth hormone; *Ghr*: growth hormone receptor; *Igf1*: insulin-like growth factor 1; *Igfbp3*: insulin-like growth factor binding protein 3; *PPARg*: peroxisome proliferator-activated receptor gamma; *Prl*: prolactin; *Tshb*: thyroid stimulating hormone b; TSS: transcriptional start site.

corroborated by lower serum IGF-1 levels in these animals ($P < 0.05$, Figure 5B). Interestingly, mRNA expression of hypothalamic growth hormone releasing hormone (*Ghrh*) as well as hypophyseal growth hormone (*Gh*) were normal, and transcripts of other pituitary hormones that can contribute to reduced body length in mice such as prolactin (*Prl*) or thyroid stimulating hormone beta (*Tshb*) were not changed (Figure 5D and S1C). Likewise, hepatic deiodinase 1 (*Dio1*) and serum total T4 levels of control and prazosin offspring were similar (Figure 5C,E). Since hepatic *Igf1* mRNA expression was also unaltered (Figure 5E), indicating normal IGF-1 production, we speculated that the lower serum IGF-1 concentrations could be the result of reduced secretion. And indeed, we found a significant reduction in liver growth hormone receptor mRNA expression (*Ghr*, $P < 0.01$, Figure 5E), providing a molecular explanation for the reduced serum IGF-1 concentrations. We subsequently analyzed hepatic DNA methylation at important regulatory regions within the *Igf1* and *Ghr* gene (Figure 5F–H), and identified a CpG site in an important regulatory region close to the transcriptional start site of the *Ghr* gene that was differentially methylated between offspring of wildtype and prazosin mothers ($P < 0.05$, Figure 5H). Most remarkably, the hepatic mRNA expression of *Ghr* was significantly negatively correlated to the percentage of methylated DNA at this position in all animals (Figure 5I), indicating a direct role of this specific CpG site in the regulation of *Ghr* mRNA transcription. This effect was tissue-specific and not observed in gWAT (Figure S1G).

4. DISCUSSION

Our data identify a novel role for maternal α1-adrenergic signaling in fetal programming of endocrine and metabolic function. We reveal that α1-adrenergic blockade by prazosin treatment during pregnancy leads to dwarfism and insulin resistance in the adult male offspring, accompanied by subsequent changes in the central regulation of thermogenesis, including a fever-like elevation of body temperature. These alterations are likely caused by altered fetal programming of the GH/IGF-1 axis at the level of the *Ghr* gene, as we identified a specific CpG site where a strong negative correlation between DNA methylation and mRNA expression is observed. Our results demonstrate that maternal α1-adrenergic blockade can be a previously unrecognized non-genetic cause for the development of a dwarfism and insulin resistance phenotype in male offspring.

4.1. Manipulation of maternal thermogenesis and metabolism

Several studies show that excessive gestational weight gain is associated with an increased risk for the development of maternal and neonatal obesity postpartum, thereby contributing to the growing health problem of obesity (reviewed in [29,30]). Likewise, gestational weight also has long-lasting effects, as 6–8 months postpartum, the body weight of mothers is usually 1–2 kg higher than preconceptionally (reviewed in [30]). Therefore, gestational body weight gain is closely linked to healthy offspring development, and severe alterations in maternal metabolism are likely to affect the epigenetic metabolic setpoint of the offspring.

In our study, we used the artificial uncoupler DNP, which has been reported to induce a massive increase in whole body thermogenesis and thereby metabolism [28]. However, DNP had only minor effects on maternal thermogenesis and metabolism, probably due to the relatively low dose that was used to avoid the massive reduction in food intake associated with higher drug concentrations [28], or the endogenously increased metabolic rate during pregnancy. Consequently, we did not observe IUGR, as DNP and control offspring displayed similar body weights at birth. Interestingly, male offspring gained less weight after P6. This effect was only transient and possibly caused by carry-over of DNP from the mother to the offspring [31]. Alternatively, given that DNP treatment during lactation has been associated with reduced postnatal body weight gain in rats [32], one could speculate that caloric restriction postnatally might contribute; however, this would likely also affect female offspring, which we did not observe. Moreover, a placental transition of DNP also explains the higher thermogenic capacity of the offspring at P5. Likewise, our treatment with prazosin, a peripheral α1-adrenergic antagonist, evoked maternal thermogenesis. As part of its anti-hypertensive actions, prazosin also competitively antagonizes the contractile response of peripheral vessels induced by norepinephrine, thereby enforcing vasodilation of the tail artery and heat loss, which subsequently triggers compensatory thermogenesis [27]. This was indeed observed in the pregnant females; however, iBAT thermogenesis was less elevated than in adult males treated with prazosin [27], probably a consequence of the reduced BAT activity during pregnancy [33,34]. In contrast to DNP treatment, maternal prazosin intervention had severe consequences for the male offspring lasting into adulthood; however, since the effects were markedly different from those observed with DNP, it can be concluded that they are likely not the result of elevated maternal thermogenesis, but rather a more direct consequence of maternal α1-adrenergic blockade. Nevertheless, despite the low passage of prazosin across the placenta [35], direct effects can also not be fully excluded. Likewise, although the prazosin treatment was stopped at birth, it might still affect subsequent maternal care or lactation; however, lactational alterations are usually associated with offspring obesity [36] or reduced *Gh* mRNA expression [37], which was not observed in our animal model.

4.2. Phenotype of the offspring from mothers treated with prazosin

The most obvious phenotype of the prazosin offspring was their reduced body length, indicative of dwarfism. Dwarfism is usually caused by mutations in key regulators of the GH/IGF-1 axis (reviewed in [38]), and several dwarf mice have been characterized to date [39]. To our knowledge, this study is the first to show that altered fetal programming of hepatic *Ghr* expression can be an underlying epigenetic cause for dwarfism in mice. Most importantly, we identified a specific CpG in the alternative transcriptional start site of the *Ghr* gene [40], which was hypermethylated in the livers of prazosin offspring. Intriguingly, the methylation of this CpG significantly correlated negatively to hepatic but not gWAT *Ghr* mRNA expression in all animals, suggesting that it has an important role in the fetal programming of hepatic GHR signaling. Therefore, our data suggest that maternal interventions affecting α1-adrenergic signaling permanently reprogram the GH/IGF-1 axis, affecting not only body length, but also food intake, insulin sensitivity and body temperature regulation.

The metabolic alterations in prazosin offspring are somewhat complex. While GH deficiency is usually associated with improved insulin sensitivity in mice [41], lower IGF-1 serum levels correlate with insulin resistance [42], suggesting that not all effects of growth hormones on glucose metabolism are mediated by IGF-1. In humans, GH deficiency in children is often accompanied by higher insulin sensitivity, while adults are frequently insulin resistant [43,44]. Interestingly, mice with a ubiquitous deletion of GHR from birth or in adults maintain their elevated insulin sensitivity despite lower IGF-1 levels [45]; only a liver specific GHR knockout displays insulin resistance [46]. However, when this knockout occurs in adult animals, glucose tolerance again remains unaffected [47]. In addition, alterations of the pulsatile release pattern of GH, e.g. by inactivating ghrelin-O-acyltransferase [48], constitute another level of IGF-1 regulation. Taken together, these studies

underline that site and onset of growth hormone insensitivity play a crucial role for the metabolic phenotype. Our mouse model best resembles the inborn liver-specific deletion of GHR causing insulin resistance, which concurs well with a possible epigenetic fetal programming mechanism of the hepatic GHR instituted during embryonal development.

Likewise, the relationship between GH signaling and body temperature regulation is difficult to interpret, and conflicting findings have been reported. While GHRH knockout mice display higher body temperature [49], lower body temperature is found in a mouse model of growth hormone deficiency [50]. With regard to brown fat thermogenesis, no effect of growth hormone was observed in wildtype mice on *Ucp1* mRNA expression [51]. The situation is complicated by the fact that several other mouse models of dwarfism, such as the Ames mice, also display alterations in TSH or serum thyroid hormone concentrations [52], which additionally modulate body temperature regulation, but this was not observed in our paradigm. Thus, the thermoregulatory phenotype of our prazosin offspring is best described as mild pyrexia with hallmarks of elevated body temperature, normal brown adipose tissue thermogenesis, and unaltered UCP1 protein expression as well as inappropriately low heat dissipation over the tail and reduced food intake [53], which, however, was not the consequence of altered hypothalamic *Npy*, *Pomc*, *Agrp*, or orexin gene expression. Interestingly, elevated temperature was already noticeable in prazosin offspring shortly after birth, resembling the excessive sweating of children with Silver-Russell syndrome [54], a condition also characterized by dwarfism, lower IGF-1 levels, and reduced food intake [55], which can be caused by epigenetic alterations [56].

4.3. Use of α1-adrenergic antagonists in human pregnancy

Chronic hypertension, preeclampsia, and gestational hypertension affect 5–10% of all pregnancies and increase the risk of adverse pregnancy outcomes [19,23]. Accordingly, these conditions require careful monitoring and immediate and adequate treatment. Unfortunately, drug treatment options are limited, as many standard antihypertensive drugs are contraindicated during pregnancy, including angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs), or have not been adequately evaluated in pregnant women [19,23,24]. In current guidelines α-methyldopa, labetalol (α1/β-adrenergic blocker), hydralazine and nifedipine (a 1,4-dihydropyridine calcium channel blocker) are recommended as first or second-line agents in the treatment of pregnancy associated hypertension, being considered relatively safe [19,23,24]. Although prazosin is not considered a first or second-line agent for the treatment of hypertension in pregnancy in Europe or the United States (ESC guidelines [57]) and ACOG guidelines [58], it is listed as a second-line agent in the Australian and New Zealand guidelines (SOMANZ guidelines [59,60]). However, others, like the Canadian guideline SOGC, do not recommend its use during pregnancy or only in special cases, e.g. chronic kidney disease [61,62]. Unfortunately, comprehensive studies on prazosin during pregnancy are missing [63], and available animal studies only report short term changes in uterine motility [64] and a reduction in blastocyst number [64], but no data on offspring development. In humans, only single case reports are available [65] or studies have been limited to fetal development [35], resulting in the classification that prazosin treatment during pregnancy might be safe [66,67].

5. CONCLUSION

Our data suggest that α1-adrenergic blockade during pregnancy can alter the fetal programming of the GH/IGF-1 axis, an effect that might not be recognized until later in life as the offspring were born with normal weight. Importantly, this mechanism may also apply to other compounds with partial α1-adrenergic blocking activity such as labetalol, one of the first-line agents in the treatment of maternal hypertension. Consequently, retrospective analyses correlating maternal α-blockade to possible dwarfism and insulin resistance in humans are urgently needed to re-assess the safety of these drugs in pregnancy.

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Author Contributions

R.O. and J.M. designed the research. R.O., B.H., C.G., L.H., and C.K. performed experiments. R.O., B.H., C.K., H.L., H.O., H.K., and J.M. analyzed the data. R.O. and J.M. wrote the manuscript. All authors read, modified, and approved the manuscript. The authors declare no conflict of interest. We thank Julia Resch and staff of the GTH animal facility for technical assistance. This study was funded by the Deutsche Forschungsgemeinschaft (Heisenberg Programm MI1242/2-1 and MI1242/3-1 to JM; SPP1629 "Thyroid TransAct" MI1242/6-1 to JM; Emmy Noether-Programm KI1887/2-1 to HK). HO is a Lichtenberg fellow of the Volkswagen Foundation. BH, CG, and LH are associated students of the GRK1957 "Adipocyte-Brain-Crosstalk".

CONFLICT OF INTEREST

None declared.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.molmet.2017.06.016.

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