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Maternal circadian rhythms and the programming of adult health and disease

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Abstract

The in utero environment is inherently rhythmic, with the fetus subjected to circadian changes in temperature, substrates and various maternal hormones. Meanwhile, the fetus is developing an endogenous circadian timing system, preparing for life in an external environment where light, food availability and other environmental factors change predictably and repeatedly every 24 hours. In humans, there are many situations that can disrupt circadian rhythms, including shift work, international travel, insomnias and circadian rhythm disorders (e.g., advanced/delayed sleep phase disorder), with a growing consensus that this chronodisruption can have deleterious consequences for an individual's health and wellbeing. However, the impact of chronodisruption during pregnancy on the health of both the mother and fetus is not well understood. In this review we outline circadian timing system ontogeny in mammals, and examine emerging research from animal models demonstrating long term negative implications for progeny health following maternal chronodisruption during pregnancy.

Keywords: circadian rhythm, pregnancy, programming, metabolism

Circadian rhythms

Circadian rhythms evolved as an adaptation to the predictable changes in day and night, and ensure aspects of behaviour and physiology are timed to occur at the most appropriate part of the day-night cycle. The three critical features of the circadian timing system are 1) a central pacemaker capable of generating endogenous circadian rhythms, 2) the capacity to integrate environmental signals to ensure circadian rhythms are appropriately synchronised to the environment, and 3) efferent pathways from the pacemaker to the periphery to orchestrate whole body circadian rhythmicity.

In mammals, the central pacemaker is the bilateral suprachiasmatic nucleus (SCN) of the hypothalamus, a group of approximately 10,000 cells lying on either side of the third ventricle, immediately dorsal to the optic chiasm (58). The basis of circadian rhythm generation is a molecular transcription-translation feedback loop involving a series of core-clock genes. Molecular rhythm generation has been extensively reviewed elsewhere (55). In brief, CLOCK and BMAL1 proteins form a heterodimer to drive transcription of *Per1/2/3* and *Cry1/2* genes. Translation, accumulation and post-translational modifications allow these genes to feedback to repress their own transcription. Simultaneously, CLOCK/BMAL1 heterodimers drive transcription of *Rev-erbα/β* and *Rorα/β/γ* genes, which in turn suppress or induce, respectively, the expression of *Bmal1* through ROR response elements. Together these two interlocked loops result in the 24 hour rhythms of clock gene expression. Regulatory mechanisms act to support and fine-tune the molecular feedback loops through protein modifications including phosphorylation, ubiquitination, acetylation and sumoylation (30). Rhythmic information is transmitted to the rest of the cell through clock-controlled genes, many of which are themselves transcription factors, thereby amplifying the rhythmic signal throughout the transcriptome. As a result, up to 10% of the transcriptome (2), and 20% of the proteome (67) oscillates predictably across 24 hours.

The second critical feature of the circadian system is that rhythms can be entrained to the external environment. Light information, which is perceived by the retina and transmitted through a direct neural pathway to the central SCN clock, is the predominant zeitgeber (time giver). Other signals including activity, food intake, timing of sleep, and stress/arousal are also transmitted to the SCN and alter the

timing of rhythmicity (12, 57, 59). This capacity for entrainment is critical for ensuring organisms remain aligned to a changing external environment.

Finally, the SCN signals to the rest of the brain and periphery through neural and hormonal pathways. Neuroanatomical tracing studies have shown that neurons of the SCN primarily project to a few hypothalamic nuclei, including the paraventricular nucleus (PVN), the medial preoptic area (MPOA) and the dorsomedial nucleus (DMH) (33). This allows propagation of the signal from the SCN through its contacts with the neuro-endocrine neurons of the hypothalamus. For example, rhythmic melatonin secretion from the pineal gland is controlled by a multi-synaptic SCN-PVN-superior cervical ganglion-pineal pathway (83). The hypothalamic-pituitary-adrenal axis is also controlled by the SCN indirectly through gamma-aminobutyric acid (GABA)-ergic interneurons in the subPVN and the DMH, which in turn inhibit corticotropin-releasing hormone (CRH)-containing neurons in the PVN (34). Together these multi-synaptic pathways originating in the SCN control the rhythmic release of melatonin and cortisol/corticosterone, which in turn signal time of day information to target tissues via melatonin and glucocorticoid receptors expressed throughout the periphery. The connections from SCN neurons to preautonomic neurons within the hypothalamus can also regulate both sympathetic and parasympathetic outflow to peripheral organs, including the pancreas, adrenal and liver, generating rhythms in circulating concentrations of glucose, insulin and free fatty acids (9, 42).

The importance of secreted factors, which complement actions of neural pathways in controlling circadian rhythms, was elegantly demonstrated by Silver and co-authors. In their studies, SCN tissue transplanted into SCN-ablated hamsters was able to restore rhythms of running activity, despite the prevention of neural outgrowth by a semipermeable membrane surrounding the transplanted tissue (82). Factors secreted from the SCN, including vasopressin, TNF-α, prokineticin-2, and cardiotrophin-like cytokine, each alter locomotor activity (13, 40, 41). Together these output mechanisms result in a highly regulated, multi-level circadian timing system that orchestrates physiological functions so that they occur at optimal times relative to the environment.

Ontogeny of the fetal circadian timing system

The development of the circadian timing system is a gradual process, beginning in utero with SCN neurogenesis, followed by synaptogenesis between SCN neurons, innervation from the retino-hypothalamic tract (RHT), development of efferent connections, and finally the emergence of a mature circadian system displaying overt physiological rhythms. There are significant inter-species differences in the timing of this process, with several key components occurring postnatally in species such as rats and mice that are born relatively immature, in contrast to prenatal emergence of similar developmental milestones in sheep and primates, including humans (Figure 1).

SCN formation and RHT innervation

In rats, SCN neurogenesis occurs from embryonic day 14 to 17 (E4-17, 64-77% of term gestation) (58). Synaptogenesis occurs gradually from E21 (95% of term) to postnatal day 2 (P2), then proceeds rapidly from P2 to P6. However, it is not until P10 that synaptic density matches adult levels (58). RHT development occurs from E21 through to P15 (58), with light responsiveness, as measured by *c-fos* induction, appearing at P1 (44).

In sheep, the SCN is detectable at E52 (35% gestation), although it is small in terms of cell volume and number at this stage. By E58 (39% of term) the SCN has reached its full number of neurons (90), and the projections from the RHT first appear at this stage, with gradual innervation continuing. By E121 (82% of term) the pattern of retinal innervation to the SCN is consistent with the adult (90). This would suggest that the lamb SCN is responsive to light at birth, although this has not been evaluated in published literature.

In humans it is difficult to determine the precise stage of SCN development due to limited availability of suitable material. However, clear vasopressin staining, a marker for the localisation of the SCN, can be detected in the human fetus from 31 weeks of gestation, ~78% of term (88). In squirrel monkeys, SCN neurogenesis occurs between E27 and E48 (16-29% of term). The SCN is innervated by the RHT and is responsive to light exposure at birth in baboon infants (74).

Development of circadian rhythmicity

The development of SCN rhythmicity has been well characterised in rodents. Rhythms in glucose utilisation, vasopressin mRNA expression and neuronal firing rate are detectable in the fetal rat SCN in the days leading up to birth (71, 73, 80). Core-clock gene mRNA is detectable in whole mouse embryos from E10 (20), and in the SCN from E19 (85), yet rhythmicity of expression may not appear until after birth (85). Similarly, molecular clocks in peripheral tissues develop slowly through the late prenatal and early postnatal period. Microarray studies on fetal liver collected from mice at E18-19 reveal little evidence of rhythmic core-clock gene expression (48), consistent with previous reports in a variety of peripheral tissues (20, 85). In our own studies in rats, we found that while *Bmal1* and *Per1* expression was constitutive, *Per2* was rhythmically expressed (2-fold amplitude) in fetal liver at E19-20 (93). Importantly, *Per2* expression in fetal liver responded to disrupted external photoperiod, suggesting it is regulated by maternal factors rather than CLOCK and BMAL1 at this stage of development. Unlike other peripheral tissues which are arrhythmic at this stage, the fetal adrenals express robust antiphase rhythms of *Per2* and *Bmal1* at E18, with an accompanying rhythmic secretion of corticosterone (53, 91). However, behavioural, endocrine and molecular rhythms developing gradually after birth, with adult-like rhythmicity apparent around the time of weaning (84).

In sheep, markers of neuronal activity within the SCN become rhythmic from E90, or 62% of term (7), and rhythmic vasopressin can be detected in fetal cerebrospinal fluid at E108 (75% of term, 86). In the last third of pregnancy, biophysical variables including heart rate and breathing can be detected and change predictably over 24 hours (17). Rhythmic changes in melatonin, cortisol and prolactin are present in fetal plasma at E120 (51, 106), although the rhythm in melatonin is due to maternal secretion (50).

In non-human primates, rhythms of glucose utilisation as well as *Bmal1* and *Per2* mRNA expression become detectable in the SCN at 90% of term (70, 92). In human fetuses, heart rate and fetal movement rhythms are absent at week 13 of gestation but readily detectable by week 20 (101). As illustrated by this data and summarised in Figure 1, the circadian system of sheep and primates reaches a later stage

of development in utero than that of rodents. However, newborn lambs and primates mostly do not show circadian rhythmicity of endocrine outputs. The first evidence for rhythmic cortisol secretion in human infants appears at 3 weeks of age (96), and melatonin rhythms emerge 9 weeks after birth (38). The prenatal establishment of endogenous circadian rhythms in these species is evident, however, by rhythms of temperature, which are detectable in both sheep and primates immediately following birth (8, 56, 77, 78).

Entrainment of fetal rhythmicity

The developing fetal circadian system can respond to changes in the external environment, even in species whose rhythms develop relatively late in gestation. For example, fetal glucose utilisation rhythms in rats respond to phase shifts of the external photoperiod (69). The maternal signals that drive this response are likely multi-factorial. A prime candidate for maternal entrainment of fetal rhythmicity is melatonin, which crosses the placenta (76) and can bind to melatonin receptors expressed on most fetal tissue (105). Timed melatonin application can phase shift both core-clock and steroidogenic acute regulatory protein (*StAR*) gene expression in cultured rat fetal adrenals (91). Furthermore, nocturnal melatonin administration to rat dams can entrain rhythmicity of the fetal adrenal clock following exposure to constant light (53). Transmission of an entrainment signal from mother to fetus, whether it be endocrine or metabolic, is required to pass through the placenta. Interestingly, there is preliminary evidence for a placental clock, with both the junctional and labyrinth zones showing time dependent expression of the core clock genes in the rat placenta (103). Furthermore, the glucocorticoid receptor and components of the placental glucocorticoid barrier (11b-hsd1 and Abcb1b) are also rhythmic (102), as is the expression of the melatonin receptor, MT1 (45). The role of the placenta in the entrainment of fetal rhythmicity warrants further investigation.

What is the role of the fetal molecular clock?

The mammalian fetal circadian system develops gradually during late gestation and the early postnatal period. However, it is unknown whether the absence of a fetal clock influences growth or survival in

utero. BMAL1 is a critical component of the circadian machinery, and consequently *Bmal1* null mice display complete behavioural and molecular arrhythmicity (10). Cumulative data from matings of heterozygous *Bmal1* knockout mice revealed significant divergence from the expected Mendelian distribution of offspring genotypes at weaning, such that both heterozygous and knockout genotypes are underrepresented compared to the wild type genotype (5). This suggests reduced perinatal survival of those mice carrying the *Bmal1* null mutation. Reduced weight and poorer health of the surviving *Bmal1* null mice was also apparent even at this early age (5), suggesting that the absence of a functional clock during early development has important consequences for health and mortality. Because weaning outcomes were assessed in this study, however, it was not clear at which stage of development the progeny losses and failure to thrive occurred. To address this question, we mated *Bmal1* heterozygous knockout mice and measured fetal genotype, fetal and placental weights in late gestation. In contrast to outcomes at weaning, the appropriate Mendelian ratio of genotypes was present in late gestation and there was no effect of *Bmal1* genotype on fetal or placental weights at day 17.5 of gestation (Figure 2). We can conclude that in the absence of a functioning molecular clock, *Bmal1* null fetuses can develop normally, implying that a fetal clock is not necessary for in utero growth and survival, at least in mice, where SCN rhythmicity is not evident until near term (81). Any subsequent deaths that occur prior to weaning may be due to absence of the circadian timing system during critical periods of postnatal development.

Other studies have suggested that the absence of a fetal clock may influence fetal growth and morphology. Landgraf and colleagues demonstrated that *Clock*Δ*19* mutant pups are physically different to wild types at postnatal day 0, with significantly increased fat depots, bone ossification and altered body morphology and organ size (43). Further interrogation however revealed that these changes could largely be attributed to maternal effects; when litter mates from *Clock*Δ*19* heterozygous dams were compared, the effects of genotype were reduced, with *Clock*Δ*19* mutant pups differing only from their wild type litter mates in torso size (43). It must also be kept in mind that the genes responsible for rhythm generation may be pleiotropic, and that any differences observed in fetal growth and

development may not be due to the absence of a functional clock, but rather other unknown functions of these genes.

Maternal chronodisruption and programming of progeny health

Evidence from epidemiological studies in humans has revealed associations between circadian disruption and problems with fertility and pregnancy, particularly miscarriage (23). Many peri-conceptional challenges or insults affect not only pregnancy, but also the long-term health of progeny, where the exposure alters gamete, embryonic or fetal patterns of development, likely in part an adaptive response improving fitness after birth (27). This concept that early physiological challenges can alter the developmental trajectory and hence later health of progeny is referred to as developmental programming. Mechanisms for persistent effects that have been identified in preclinical models include altered cell division as well as epigenetic processes within cells that alter subsequent gene expression (27). Effects of such challenges often depend on timing as well as severity. For example, the effects of severe famine on offspring health during the Dutch Hunger Winter of World War 2 differed depending on the stage of pregnancy when the women were malnourished (75). Rates of chronic heart disease were highest in adult offspring of women exposed in early pregnancy, whereas impaired glucose homeostasis was most evident in adults whose mothers were malnourished in late pregnancy (75). Whether maternal circadian rhythm disruption and its impact on the developing progeny impairs later health has not yet been evaluated in human studies, with the only available evidence coming from animal models.

Animal models of maternal circadian rhythm disruption

A variety of animal models have been utilised to investigate the relationship between maternal chronodisruption and long term progeny health, and each have different impacts on the adult circadian system (Table 1). For example, mice with genetic mutations or gene deletions have been used to assess the impact of maternal rhythm disruption on the developing progeny (32). Another approach is surgical SCN ablation, which physically destroys the central clock and immediately induces a loss of behavioural

and other rhythms (18, 72). Maternal chronodisruption can also be induced by altering the light cycle that dams are subjected to during pregnancy and/or lactation (54, 95). As light is the principal environmental cue for the circadian system, exposing an animal to chronic phase shifts of the photoperiod forces the animal to constantly attempt to adjust its circadian timing system, whereas exposure to constant light means that the central and peripheral molecular circadian machinery free-runs without daily entrainment by light. Maternal pinealectomy removes melatonin secretion capacity and hence the fetus no longer receives that maternal signal (24). Finally, changing the timing of maternal food access greatly disrupts maternal behaviour and the timing of substrate availability to the fetus (3).

Maternal chronodisruption and progeny circadian rhythms

As discussed above, the circadian system develops progressively throughout the prenatal and early postnatal period, and the fetus is responsive to changes in maternal rhythmicity. In adulthood, genetic factors predominantly determine endogenous circadian rhythms. This was demonstrated by Viswanathan and Davis who showed that the free running period of heterozygous *tau* mutant hamsters was 21.7 hours, despite being born to and raised by wildtype dams with a period of 24 hours (98). However, recent advances in our understanding of the highly complex nature of circadian rhythm generation including post-translational control and epigenetic regulation of the molecular clock suggest that environmental conditions during development may have a long term impact upon the circadian timing system of progeny. Indeed, a level of developmental plasticity is observed when the perinatal environment is disrupted, with mice housed in either short or long day lengths from birth until weaning continuing to display altered *Per1* gene expression in individual SCN neurons as adults (15). Whether prenatal disruption of the environment has long term impacts on circadian rhythm generation and/or entrainment is still unclear, and may differ between species depending on the timing of circadian system development.

The first studies to interrogate the role of maternal rhythmicity on the progeny utilised SCN ablation of rat and hamster dams during early pregnancy (18, 72). Despite exposure to an arrhythmic in utero environment, offspring born to SCN-ablated dams were found at weaning to display, on average, free running periods of activity and drinking similar to that of controls. Intriguingly, however, there was reduced within-litter synchrony in the timing of pup behaviour. Jud and Albrecht used a genetic approach to interrogate the postnatal development of behavioural rhythmicity in offspring exposed to an arrhythmic environment in utero (32). Female double mutant *Per1*^{Brdm1}/ *Per2*^{Brdm1} and *Per2*^{Brdm1}/*Cry1*$^{-/-}$ mice, which display behavioural arrythmicity due to absence of a functional maternal circadian clock, were mated with wild type males to generate heterozygous offspring. At 6 weeks of age, the period of wheel running activity of heterozygote offspring under constant conditions was unaffected by maternal genotype. However, similar to the SCN ablation studies, behavioural synchrony was reduced between litter-mates. Given that the animals from both studies were maintained in constant environmental conditions (constant darkness) from birth, and were reared by dams without a functional clock (due to either SCN ablation or genetic mutation), it is difficult to separate pre- and post-natal maternal influences in these studies. Nevertheless, the overall conclusion appears to be that exposure to a disrupted maternal environment in utero does not profoundly affect postnatal development of circadian rhythms, at least in rodents.

In our laboratory, we assessed temperature rhythms of male offspring from rat dams exposed to chronic phase shifts (CPS) of the photoperiod throughout gestation and for one week after birth (95). In adult progeny at 5 months of age, there was no effect of maternal CPS on the basal, minimum, maximum core body temperature or the phase (timing) of the temperature rhythm, under conditions of either 12L:12D housing or when free-running during constant darkness (95). Furthermore, the phase shift in temperature rhythms induced by a single nocturnal light pulse was similar in offspring of both control and CPS-exposed mothers (95). Again, this temperature data might suggest that maternal circadian rhythm disruption has minimal impact upon either the endogenous circadian rhythm, or the response of this rhythm to a light zeitgeber, in adult progeny. However, in another study of circadian outcomes in

CPS-exposed offspring, although temperature and activity profiles were unchanged, melatonin and corticosterone rhythms were profoundly disrupted (54). Nocturnal melatonin secretion was suppressed to levels normally seen in the day time, with unchanged low melatonin during the day, and peak corticosterone secretion occurred at the beginning of the light phase rather than early in the dark phase (54). Rhythms in heart rate were also disrupted, with continuous recording revealing a reduced mesor (rhythm-adjusted mean), greater amplitude of the rhythm, and an advance in the timing of acrophase (time of peak levels) of almost an hour (54). Tail cuff assessments of blood pressure revealed higher systolic blood pressure during the night and increased amplitude of the rhythm in CPS-exposed adult offspring (54). In contrast, core-clock and clock-controlled gene expression in hypothalamic blocks containing the SCN was only minimally affected by maternal CPS exposure (54). Rat offspring from dams exposed to constant light throughout pregnancy also displayed altered melatonin and corticosterone secretion, with nocturnal levels of both hormones significantly reduced compared to control progeny (97). However, in the latter study only two time points were assessed (1100h and 2300h), making the degree of disruption difficult to assess. Intriguingly, in progeny of rat dams exposed to constant light throughout pregnancy, the changes to offspring melatonin and corticosterone secretion were rescued by nocturnal melatonin supplementation to the dams (97), implicating maternal melatonin as a key signal that programs progeny circadian rhythms.

Maternal chronodisruption and progeny neurobehavioral and cognitive outcomes

There is growing evidence that maternal chronodisruption can impair cognitive and neurobehavioral outcomes in the progeny. Exposure of rat dams to constant light during gestation increased adult progeny escape latency in the Morris Water Maze test over consecutive days of testing, suggesting deficits in spatial memory and learning (97). These offspring also exhibited lower gene expression of glucocorticoid receptor (*Nr3c1*) and NMDA receptor subunits (*Grin1b, Grin3a, Grin3b*) in the hippocampus (97), each important components of the molecular machinery of learning. Male offspring also display increased anxiety-like behaviors including reduced time in the central area in Open Field

behavioral tests, and reduced distance travelled and reduced time in the open arms during Elevated Plus Maze testing (100). Interestingly, many of these outcomes were rescued by administration of melatonin in the dams' drinking water, suggesting melatonin may play a crucial role in mediating the negative effect of maternal circadian disruption on progeny outcomes. Studies in mice have demonstrated that even dim light at night can affect the behavioural phenotype of adult offspring. Exposure of mice dams to as little as 4 lux light at night pre- and/or postnally increased anxiety-like behaviors in the Elevated Plus and Passive Avoidance Tests in both male and female offspring at 9 weeks of age (6). Chronic phase delays, where the photoperiod was delayed by 8 hours every second day throughout pregnancy, also increased depressive-like behaviors and anhedonia in mice offspring (107). Interestingly, the behavioral phenotype of the F2 generation was also affected, but with both male and female offspring displaying anti-depressive-like behaviours in the Forced Swim Test (107). In contrast, in our own experiments, adult male offspring of CPS exposed rats were indistinguishable from controls in measures of behavioral despair and anxiety measured using the Forced Swim Test and Open Field Test, respectively (95).

Maternal chronodisruption and progeny metabolic health

Maternal chronodisruption alters metabolic health of progeny in several animal models (24, 54, 95, 97), although not universally (94). We first examined the impact of maternal rhythm disruption on the long term metabolic health of progeny in our model of maternal CPS throughout pregnancy and the first week of lactation (Figure 1, 95). Progeny of dams exposed to CPS from conception until one week after birth developed gender- and age-dependent metabolic dysfunction. Specifically, young adult 3 month-old male offspring had increased adiposity and hyperleptinaemia (95). Although they did not show impairment at 3 months of age, female offspring were hyperinsulinaemic and hyperleptinaemic and also had impaired glucose tolerance as mature adults at 12 months of age, due to reduced insulin sensitivity and despite increased glucose-stimulated insulin secretion (95). This impairment of progeny glucose tolerance after maternal CPS exposure was confirmed by Mendez and co-authors (54), who found poor glucose tolerance in young adult 3 month-old male offspring. Interestingly, in this study they exposed

the dams to CPS from conception only until day 18 of pregnancy, when the dams were returned to a 12L:12D photoperiod. This suggests the changes to glucose metabolism observed in adult offspring arise from in utero exposure to CPS rather than any environmental signals during the early postnatal period.

Maternal pinealectomy, which removes fetal exposure to maternal melatonin signals of environmental rhythms, also induces glucose intolerance in adult offspring (24). Four month old male and female progeny of pinealectomised dams display glucose intolerance, impaired glucose stimulated insulin secretion from isolated islets and hepatic insulin resistance, but no change in whole body insulin sensitivity compared to control progeny (24). Although expression and activation of insulin signalling pathways in muscle are not altered, these progeny have decreased insulin-induced Akt phosphorylation and elevated PEPCK levels in the liver (24), which would be expected to increase gluconeogenesis and impair its suppression by insulin. Changes in glucose metabolism of adult progeny from pinealectomised dams or dams exposed to constant light can also be rescued by administration of melatonin to the dams during pregnancy (24, 97).

The maternal metabolic response to circadian rhythm disruption may influence progeny outcomes. It has been well described in both animal models and human studies that chronodisruption perturbs metabolic homeostasis of the individual (reviewed in 62). The impacts of chronodisruption during pregnancy on maternal metabolism is less clear, although we have demonstrated changes to the phase and level of key metabolites and hormones during late gestation in CPS dams (93). Specifically, leptin and insulin were down-regulated, whereas metabolite profiles became out of phase (glucose, triglycerides) or arrhythmic (free fatty acids, cholesterol) during late gestation (93). Similarly, the typical high amplitude expression profiles of core-clock and metabolic genes in maternal liver was reduced (93). Exposure to CPS during pregnancy altered the timing of food consumption in the dams, leading to intermittent grazing, compared to the control pattern of consolidated bouts of feeding during the dark period (93). Poor metabolic control during pregnancy is a known risk factor for increased obesity, metabolic and cardiovascular disease of adult offspring (46). Maternal chronodisruption will

also affect sleep quality and timing, and human studies have demonstrated that poor quality and quantity of sleep is a known risk factor for gestational diabetes and hyperglycaemia (11, 21, 29). The interwoven relationship between circadian rhythms, sleep and metabolism likely contributes to the effect of chronodisruption on offspring metabolic health.

It was therefore of interest to discover that restricting food availability of pregnant rats to the inactive day period can impair metabolic outcomes in progeny (3). Exposure of rat dams to a day-restricted feeding protocol through gestation and lactation programmed poor glucose tolerance and reduced glucose stimulated insulin secretion of pancreatic islets in the male offspring (3). In that study, an analysis of cumulative food consumption revealed an 18% and 10% reduction across gestation and lactation respectively compared to *ad libitum* fed controls (3). When progeny outcomes of day-fed rats were compared to those of pair-fed animals, rather than ad libitum controls, differences in glucose metabolism were still evident (3), indicating that changes in the timing, rather than amount, of food consumption was sufficient to permanently alter the glucose metabolism of the offspring.

Thus, maternal circadian rhythm disruption during gestation impairs metabolic homeostasis in adult rat offspring in four different experimental models; chronic phase shifting, constant light, pinealectomy and day-restricted feeding. Melatonin replacement studies suggest melatonin has a role, and normalizing the maternal pattern of circulating melatonin at least partially restores progeny metabolic outcomes in several models. Interestingly, melatonin-proficient melatonin receptor knockout mice also display an adverse metabolic phenotype, suggesting lifelong absence of melatonin action negatively impacts glucose homeostasis (16). In rats under CPS conditions, only subtle changes in maternal melatonin profiles were observed when dams were assessed between 12 and 36 hours after return to a normal photoperiod (54, 93), suggesting mechanisms additional to melatonin can induce programming of progeny metabolism. However, given the dams were exposed to repeated reversals of the photoperiod throughout pregnancy, the profile of melatonin secretion in the CPS dams is likely to deviate from the controls at other periods not assessed in our study.

As discussed above, prenatal exposures to adverse events can have a lifelong effect upon later body composition and metabolic homeostasis. Birth weight can be used as a proxy for intrauterine growth, with both human and animal studies demonstrating a relationship between small size at birth and perturbed glucose metabolism in adulthood (25, 64). A logical question therefore is whether intrauterine growth restriction (IUGR) mediates the impact of maternal chronodisruption on offspring metabolic homeostasis? An analysis of the models of chronodisruption reported in this review reveal that maternal constant light exposure and day-restricted feeding during pregnancy can impair fetal growth (3, 53), and IUGR may therefore contribute to the observed changes to metabolic homeostasis of adult offspring in these models. However, in other studies demonstrating a perturbed metabolic phenotype of adult offspring after maternal constant light, birthweight was unchanged (97). Maternal pinealectomy did not alter progeny weight, although pups were not weighed immediately following birth (24). Similarly, we and others find no evidence of IUGR following maternal CPS exposure when assessed at late gestation (93) or at birth (54, 95). This suggests that in these models of maternal chronodisruption, the changes to progeny metabolic homeostasis are not due to IUGR. Intriguingly, Mendez and colleagues found in their studies that CPS-exposed offspring were in fact born significantly heavier (+14%), likely due to an increase in gestation length of ~12 hours (54). We also found a trend for increased gestation length in rats exposed to CPS throughout pregnancy (95), although this was not significant, possibly due to the once-daily monitoring in our studies compared to the constant monitoring by Mendez and colleagues. In rodents, the timing of birth is controlled by photoperiod (49), and regulated by uterine clocks (66), and this may explain the changes to gestation length observed following CPS exposure. In turn, the longer gestations may contribute to the lower melatonin secretion observed in the adult offspring (54). We have previously reported that overnight urinary excretion of the melatonin metabolite 6-sulphatoxymelatonin in 20 year-old men and women is negatively correlated with their gestation length at birth, indicating that as gestation length increases to postmaturity, nocturnal melatonin secretion of the individuals as adults is reduced (35). This suggests that in both rodent and human pregnancies there are intriguing, yet not well understood, relationships between gestational and postnatal timing systems.

Nevertheless, maternal circadian rhythm disruption does not always program metabolic homeostasis in progeny. Recently, we used *Clock*Δ*19* mutant dams to evaluate the absence of peripheral organ rhythmicity in the mother on long term glucose homeostasis of heterozygous offspring (94). By mating a strain of melatonin proficient *Clock*Δ*19* mutant dams with wild type males (and vice versa), we created heterozygous offspring (that express circadian rhythms in their own tissues) exposed to a non-rhythmic in utero environment. There were only minimal changes to metabolic homeostasis of the adult offspring of *Clock*Δ*19* dams compared to heterozygotes gestated by wild-type dams, although there were non-significant trends for hyperleptinaemia and hyperinsulinaemia. One explanation for the lack of effect may be that the 12L:12D photoperiod both groups of mothers were housed in was sufficient to sustain relatively normal profiles of activity and melatonin secretion in the *Clock*Δ*19* mutant dams (39). Under these conditions, although the mutation produces dampening of peripheral core-clock gene expression (37), some aspects of central circadian rhythmicity, including melatonin secretion, are entrained by environmental cues and may reduce impact upon the fetuses.

Conclusion

A growing body of evidence from animal models suggests a relationship between maternal chronodisruption and long term offspring health. In particular, circadian disruption during pregnancy programs changes in offspring circadian, endocrine and metabolic function into adulthood. What is the relevance of these studies to human pregnancies? There are many situations that can cause circadian disruption in humans, but perhaps the most prevalent is shift work. A high proportion of the workforce is engaged in shift work (between 15 and 20%), and many women shift workers are of reproductive age (4). Although the number of pregnant women who work shifts is currently unknown, there is no evidence to suggest that women immediately cease shift work upon becoming pregnant. Systematic reviews of epidemiological studies suggest at most weak relationships between shift work during pregnancy and gestation length or birthweight, although there is accumulating evidence for increased rates of miscarriage suggesting disrupted pregnancy recognition and/or fetal development (23, 52, 61).

However, we are not aware of any human studies that have assessed the long term health of offspring born to mothers who worked shifts during pregnancy. Until such time, assessing impacts of maternal chronodisruption on progeny must rely on animal studies.

Studies in mice and rats have provided insights into the impact of maternal circadian rhythm disruption on progeny health, summarised in Table 2. However, while there is growing evidence that maternal chronodisruption programs progeny health, there are still significant gaps in knowledge; very few studies have considered outcomes in both males and female offspring, assessed offspring throughout the life course, or evaluated a range of progeny outcomes. There are also some reservations around the use of rodents to model chronodisruption and shift work during pregnancy in humans. Rodents are nocturnal, give birth to litters, have a short gestation period, and as described above, have offspring with poorly developed rhythmicity at birth. Furthermore, because of their short gestation and relative immaturity at birth, studies in rodents to determine stages of pregnancy most susceptible to shift work will not be easily transferrable to humans. Therefore studies in animal models for which the gestation length and degree of prenatal development are more similar to human pregnancy are required. Sheep seem the logical choice, particularly given their use in a wide range of developmental programming studies (19).

Perspectives and Significance

Defining the impact of maternal chronodisruption on both progeny and pregnancy outcomes requires prospective and retrospective studies in human populations, alongside studies in animal models using a wide range of experimental approaches. Together, these will allow critical periods and underlying mechanisms to be defined, and testing of interventions to improve outcomes.

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Author contributions

T.J.V. **wrote** manuscript and prepared Figures; T.J.V., K.L.G. and D.J.K. edited and revised manuscript and approved final version of manuscript.

References

1. **Agez L, Laurent V, Guerrero HY, Pévet P, Masson-Pévet M, Gauer F**. Endogenous melatonin provides an effective circadian message to both the suprachiasmatic nuclei and the pars tuberalis of the rat. *J Pineal Res* 46: 95–105, 2009.

2. **Akhtar RA, Reddy AB, Maywood ES, Clayton JD, King VM, Smith AG, Gant TW, Hastings MH, Kyriacou CP**. Circadian cycling of the mouse liver transcriptome, as revealed by cDNA microarray, is driven by the suprachiasmatic nucleus. *Curr Biol* 12: 540–550, 2002.

3. **De Almeida Faria J, de Araújo Ferreira TM, Mancuso RI, Meulman J, da Silva Ferreira D, Batista TM, Vettorazzi JF, da Silva PM, Rodrigues SC, Kinote A, Carneiro EM, Bordin S, Anhê GF**. Day-restricted feeding during pregnancy and lactation programs glucose intolerance and impaired insulin secretion in male rat offspring. *Acta Physiol (Oxf)* 217: 240–253, 2016.

4. **Australian Bureau of Statistics**. Working time arrangements in Australia. cat no. 6342.0, 2012.

5. **Boden MJ, Varcoe TJ, Voultsios A, Kennaway DJ**. Reproductive biology of female Bmal1 null mice. *Reproduction* 139: 1077–1090, 2010.

6. **Borniger JC, McHenry ZD, Abi Salloum BA, Nelson RJ**. Exposure to dim light at night during early development increases adult anxiety-like responses. *Physiol Behav* 133: 99–106, 2014.

7. **Breen S, Rees S, Walker D**. The development of diurnal rhythmicity in fetal suprachiasmatic neurons as demonstrated by fos immunohistochemistry. *Neuroscience* 74: 917–926, 1996.

8. **Bueno C, Menna-Barreto L**. Development of sleep/wake, activity and temperature rhythms in newborns maintained in a neonatal intensive care unit and the impact of feeding schedules. *Infant Behav Dev* 44: 21–28, 2016.

9. **Buijs RM, la Fleur SE, Wortel J, Van Heyningen C, Zuiddam L, Mettenleiter TC, Kalsbeek A, Nagai K, Niijima A**. The suprachiasmatic nucleus balances sympathetic and parasympathetic output to peripheral organs through separate preautonomic neurons. *J Comp Neurol* 464: 36–48, 2003.

10. **Bunger MK, Wilsbacher LD, Moran SM, Clendenin C, Radcliffe LA, Hogenesch JB, Simon MC, Takahashi JS, Bradfield CA**. Mop3 is an essential component of the master circadian pacemaker in mammals. *Cell* 103: 1009–1017, 2000.

11. **Cai S, Tan S, Gluckman PD, Godfrey KM, Saw S-M, Teoh OH, Chong Y-S, Meaney MJ, Kramer MS, Gooley JJ, GUSTO study group**. Sleep quality and nocturnal sleep duration in pregnancy and risk of gestational diabetes mellitus. *Sleep* 40, 2017.

12. **Challet E, Mendoza J**. Metabolic and reward feeding synchronises the rhythmic brain. *Cell Tissue Res* 341: 1–11, 2010.

13. **Cheng MY, Bullock CM, Li C, Lee AG, Bermak JC, Belluzzi J, Weaver DR, Leslie FM, Zhou Q-Y**. Prokineticin 2 transmits the behavioural circadian rhythm of the suprachiasmatic nucleus. *Nature* 417: 405–410, 2002.

14. **Cheung PW, McCormack CE**. Failure of pinealectomy or melatonin to alter circadian activity rhythm of the rat. *Am J Physiol* 242: R261–4, 1982.

15. **Ciarleglio CM, Axley JC, Strauss BR, Gamble KL, McMahon DG**. Perinatal photoperiod imprints the circadian clock. *Nat Neurosci* 14: 25–27, 2011.

16. **Contreras-Alcantara S, Baba K, Tosini G**. Removal of melatonin receptor type 1 induces insulin resistance in the mouse. *Obesity (Silver Spring)* 18: 1861–1863, 2010.

17. **Dalton KJ, Dawes GS, Patrick JE**. Diurnal, respiratory, and other rhythms of fetal heart rate in lambs. *Am J Obstet Gynecol* 127: 414–424, 1977.

18. **Davis FC, Gorski RA**. Development of hamster circadian rhythms: role of the maternal suprachiasmatic nucleus. *J Comp Physiol A* 162: 601–610, 1988.

19. **Dickinson H, Moss TJ, Gatford KL, Moritz KM, Akison L, Fullston T, Hryciw DH, Maloney CA, Morris MJ, Wooldridge AL, Schjenken JE, Robertson SA, Waddell BJ, Mark PJ, Wyrwoll CS, Ellery SJ, Thornburg KL, Muhlhausler BS, Morrison JL**. A review of fundamental principles for animal models of DOHaD research: an Australian perspective. *J Dev Orig Health Dis* 7: 449–472, 2016.

20. **Dolatshad H, Cary AJ, Davis FC**. Differential expression of the circadian clock in maternal and embryonic tissues of mice. *PLoS ONE* 5: e9855, 2010.

21. **Facco FL, Grobman WA, Reid KJ, Parker CB, Hunter SM, Silver RM, Basner RC, Saade GR, Pien GW, Manchanda S, Louis JM, Nhan-Chang C-L, Chung JH, Wing DA, Simhan HN, Haas DM, Iams J, Parry S, Zee PC**. Objectively measured short sleep duration and later sleep midpoint in pregnancy are associated with a higher risk of gestational diabetes. *Am J Obstet Gynecol* 0, 2017.

22. **De Farias T da SM, de Oliveira AC, Andreotti S, do Amaral FG, Chimin P, de Proença ARA, Leal FLT, Sertié RAL, Campana AB, Lopes AB, de Souza AH, Cipolla-Neto J, Lima FB**. Pinealectomy interferes with the circadian clock genes expression in white adipose tissue. *J Pineal Res* 58: 251–261, 2015.

23. **Fernandez RC, Marino JL, Varcoe TJ, Davis S, Moran LJ, Rumbold AR, Brown HM, Whitrow MJ, Davies MJ, Moore VM**. Fixed or rotating night shift work undertaken by women: implications for fertility and miscarriage. *Semin Reprod Med* 34: 74–82, 2016.

24. **Ferreira DS, Amaral FG, Mesquita CC, Barbosa APL, Lellis-Santos C, Turati AO, Santos LR, Sollon CS, Gomes PR, Faria JA, Cipolla-Neto J, Bordin S, Anhê GF**. Maternal melatonin programs the daily pattern of energy metabolism in adult offspring. *PLoS ONE* 7: e38795, 2012.

25. **Hales CN, Barker DJ, Clark PM, Cox LJ, Fall C, Osmond C, Winter PD**. Fetal and infant growth and impaired glucose tolerance at age 64. *BMJ* 303: 1019–1022, 1991.

26. **Hamaguchi Y, Tahara Y, Hitosugi M, Shibata S**. Impairment of circadian rhythms in peripheral clocks by constant light is partially reversed by scheduled feeding or exercise. *J Biol Rhythms* 30: 533–542, 2015.

27. **Hanson MA, Gluckman PD**. Early developmental conditioning of later health and disease: physiology or pathophysiology? *Physiol Rev* 94: 1027–1076, 2014.

28. **Hara R, Wan K, Wakamatsu H, Aida R, Moriya T, Akiyama M, Shibata S**. Restricted feeding entrains liver clock without participation of the suprachiasmatic nucleus. *Genes Cells* 6: 269–278, 2001.

29. **Herring SJ, Nelson DB, Pien GW, Homko C, Goetzl LM, Davey A, Foster GD**. Objectively measured sleep duration and hyperglycemia in pregnancy. *Sleep Med* 15: 51–55, 2014.

30. **Hirano A, Fu Y-H, Ptáček LJ**. The intricate dance of post-translational modifications in the rhythm of life. *Nat Struct Mol Biol* 23: 1053–1060, 2016.

31. **Honma KI, Hiroshige T**. Internal synchronization among several circadian rhythms in rats under constant light. *Am J Physiol* 235: R243–9, 1978.

32. **Jud C, Albrecht U**. Circadian rhythms in murine pups develop in absence of a functional maternal circadian clock. *J Biol Rhythms* 21: 149–154, 2006.

33. **Kalsbeek A, Buijs RM**. Output pathways of the mammalian suprachiasmatic nucleus: coding circadian time by transmitter selection and specific targeting. *Cell Tissue Res* 309: 109–118, 2002.

34. **Kalsbeek A, van der Spek R, Lei J, Endert E, Buijs RM, Fliers E**. Circadian rhythms in the hypothalamo-pituitary-adrenal (HPA) axis. *Mol Cell Endocrinol* 349: 20–29, 2012.

35. **Kennaway DJ, Flanagan DE, Moore VM, Cockington RA, Robinson JS, Phillips DIW**. The impact of fetal size and length of gestation on 6-sulphatoxymelatonin excretion in adult life. *J Pineal Res* 30: 188–192, 2001.

36. **Kennaway DJ, Owens JA, Voultsios A, Boden MJ, Varcoe TJ**. Metabolic homeostasis in mice with disrupted Clock gene expression in peripheral tissues. *Am J Physiol Regul Integr Comp Physiol* 293: R1528–37, 2007.

37. **Kennaway DJ, Owens JA, Voultsios A, Varcoe TJ**. Functional central rhythmicity and light entrainment, but not liver and muscle rhythmicity, are Clock independent. *Am J Physiol Regul Integr Comp Physiol* 291: R1172–80, 2006.

38. **Kennaway DJ, Stamp GE, Goble FC**. Development of melatonin production in infants and the impact of prematurity. *J Clin Endocrinol Metab* 75: 367–369, 1992.

39. **Kennaway DJ, Voultsios A, Varcoe TJ, Moyer RW**. Melatonin and activity rhythm responses to light pulses in mice with the Clock mutation. *Am J Physiol Regul Integr Comp Physiol* 284: R1231–40, 2003.

40. **Kramer A, Yang FC, Snodgrass P, Li X, Scammell TE, Davis FC, Weitz CJ**. Regulation of daily locomotor activity and sleep by hypothalamic EGF receptor signaling. *Science* 294: 2511–2515, 2001.

559 41. **Kraves S, Weitz CJ**. A role for cardiotrophin-like cytokine in the circadian control of
560 mammalian locomotor activity. *Nat Neurosci* 9: 212–219, 2006.

561 42. **Kumar Jha P, Challet E, Kalsbeek A**. Circadian rhythms in glucose and lipid metabolism in
562 nocturnal and diurnal mammals. *Mol Cell Endocrinol* 418 Pt 1: 74–88, 2015.

563 43. **Landgraf D, Achten C, Dallmann F, Oster H**. Embryonic development and maternal
564 regulation of murine circadian clock function. *Chronobiol Int* 32: 416–427, 2015.

565 44. **Leard LE, Macdonald ES, Heller HC, Kilduff TS**. Ontogeny of photic-induced c-fos
566 mRNA expression in rat suprachiasmatic nuclei. *Neuroreport* 5: 2683–2687, 1994.

567 45. **Lee CK, Moon DH, Shin CS, Kim H, Yoon YD, Kang HS, Lee BJ, Kang SG**. Circadian
568 expression of Mel1a and PL-II genes in placenta: effects of melatonin on the PL-II gene expression in
569 the rat placenta. *Mol Cell Endocrinol* 200: 57–66, 2003.

570 46. **Lehnen H, Zechner U, Haaf T**. Epigenetics of gestational diabetes mellitus and offspring
571 health: the time for action is in early stages of life. *Mol Hum Reprod* 19: 415–422, 2013.

572 47. **Lewy AJ, Tetsuo M, Markey SP, Goodwin FK, Kopin IJ**. Pinealectomy abolishes plasma
573 melatonin in the rat. *J Clin Endocrinol Metab* 50: 204–205, 1980.

574 48. **Li C, Yu S, Zhong X, Wu J, Li X**. Circadian rhythms of fetal liver transcription persist in the
575 absence of canonical circadian clock gene expression rhythms in vivo. *PLoS ONE* 7: e30781, 2012.

576 49. **Lincoln DW, Porter DG**. Timing of the photoperiod and the hour of birth in rats. *Nature* 260:
577 780–781, 1976.

578 50. **McMillen IC, Nowak R**. Maternal pinealectomy abolishes the diurnal rhythm in plasma
579 melatonin concentrations in the fetal sheep and pregnant ewe during late gestation. *J Endocrinol* 120:
580 459–464, 1989.

581 51. **McMillen IC, Thorburn GD, Walker DW**. Diurnal variations in plasma concentrations of
582 cortisol, prolactin, growth hormone and glucose in the fetal sheep and pregnant ewe during late
583 gestation. *J Endocrinol* 114: 65–72, 1987.

584 52. **Van Melick MJGJ, van Beukering MDM, Mol BW, Frings-Dresen MHW, Hulshof CTJ**.
585 Shift work, long working hours and preterm birth: a systematic review and meta-analysis. *Int Arch*
586 *Occup Environ Health* 87: 835–849, 2014.

587 53. **Mendez N, Abarzua-Catalan L, Vilches N, Galdames HA, Spichiger C, Richter HG,**
588 **Valenzuela GJ, Seron-Ferre M, Torres-Farfan C**. Timed maternal melatonin treatment reverses
589 circadian disruption of the fetal adrenal clock imposed by exposure to constant light. *PLoS ONE* 7:
590 e42713, 2012.

591 54. **Mendez N, Halabi D, Spichiger C, Salazar ER, Vergara K, Alonso-Vasquez P, Carmona**
592 **P, Sarmiento JM, Richter HG, Seron-Ferre M, Torres-Farfan C**. Gestational chronodisruption
593 impairs circadian physiology in rat male offspring, increasing the risk of chronic disease.
594 *Endocrinology* 157: 4654–4668, 2016.

55. **Mendoza-Viveros L, Bouchard-Cannon P, Hegazi S, Cheng AH, Pastore S, Cheng H-YM**. Molecular modulators of the circadian clock: lessons from flies and mice. *Cell Mol Life Sci* 74: 1035–1059, 2017.

56. **Mirmiran M, Kok JH**. Circadian rhythms in early human development. *Early Hum Dev* 26: 121–128, 1991.

57. **Mistlberger RE, Skene DJ**. Social influences on mammalian circadian rhythms: animal and human studies. *Biol Rev Camb Philos Soc* 79: 533–556, 2004.

58. **Moore RY**. Development of the Suprachiasmatic Nucleus. In: *Suprachiasmatic Nucleus: The Mind's Clock*, edited by Klein DC, Moore RY, Reppert SM. Oxford University Press, 1991, p. 391–404.

59. **Mrosovsky N**. Phase response curves for social entrainment. *J Comp Physiol A* 162: 35–46, 1988.

60. **Ohta H, Yamazaki S, McMahon DG**. Constant light desynchronizes mammalian clock neurons. *Nat Neurosci* 8: 267–269, 2005.

61. **Palmer KT, Bonzini M, Harris EC, Linaker C, Bonde JP**. Work activities and risk of prematurity, low birth weight and pre-eclampsia: an updated review with meta-analysis. *Occup Environ Med* 70: 213–222, 2013.

62. **Panda S**. Circadian physiology of metabolism. *Science* 354: 1008–1015, 2016.

63. **Perreau-Lenz S, Kalsbeek A, Garidou M-L, Wortel J, van der Vliet J, van Heijningen C, Simonneaux V, Pévet P, Buijs RM**. Suprachiasmatic control of melatonin synthesis in rats: inhibitory and stimulatory mechanisms. *Eur J Neurosci* 17: 221–228, 2003.

64. **Petry CJ, Dorling MW, Pawlak DB, Ozanne SE, Hales CN**. Diabetes in old male offspring of rat dams fed a reduced protein diet. *Int J Exp Diabetes Res* 2: 139–143, 2001.

65. **Polidarová L, Olejníková L, Paušlyová L, Sládek M, Soták M, Pácha J, Sumová A**. Development and entrainment of the colonic circadian clock during ontogenesis. *Am J Physiol Gastrointest Liver Physiol* 306: G346–56, 2014.

66. **Ratajczak CK, Asada M, Allen GC, McMahon DG, Muglia LM, Smith D, Bhattacharyya S, Muglia LJ**. Generation of myometrium-specific Bmal1 knockout mice for parturition analysis. *Reprod Fertil Dev* 24: 759–767, 2012.

67. **Reddy AB, Karp NA, Maywood ES, Sage EA, Deery M, O'Neill JS, Wong GKY, Chesham J, Odell M, Lilley KS, Kyriacou CP, Hastings MH**. Circadian orchestration of the hepatic proteome. *Curr Biol* 16: 1107–1115, 2006.

68. **Remley NR, Bryon DM, Wilson FR**. The effects of pinealectomy on the activity levels in rats. *Psychon Sci* 15: 175–176, 1969.

69. **Reppert SM, Schwartz WJ**. Maternal coordination of the fetal biological clock in utero. *Science* 220: 969–971, 1983.

70. **Reppert SM, Schwartz WJ**. Functional activity of the suprachiasmatic nuclei in the fetal primate. *Neurosci Lett* 46: 145–149, 1984.

71. **Reppert SM, Schwartz WJ**. The suprachiasmatic nuclei of the fetal rat: characterization of a functional circadian clock using ^{14}C-labeled deoxyglucose. *J Neurosci* 4: 1677–1682, 1984.

72. **Reppert SM, Schwartz WJ**. Maternal suprachiasmatic nuclei are necessary for maternal coordination of the developing circadian system. *J Neurosci* 6: 2724–2729, 1986.

73. **Reppert SM, Uhl GR**. Vasopressin messenger ribonucleic acid in supraoptic and suprachiasmatic nuclei: appearance and circadian regulation during development. *Endocrinology* 120: 2483–2487, 1987.

74. **Rivkees SA, Hofman PL, Fortman J**. Newborn primate infants are entrained by low intensity lighting. *Proc Natl Acad Sci U S A* 94: 292–297, 1997.

75. **Roseboom TJ, van der Meulen JH, Ravelli AC, Osmond C, Barker DJ, Bleker OP**. Effects of prenatal exposure to the Dutch famine on adult disease in later life: an overview. *Mol Cell Endocrinol* 185: 93–98, 2001.

76. **Schenker S, Yang Y, Perez A, Acuff RV, Papas AM, Henderson G, Lee MP**. Antioxidant transport by the human placenta. *Clin Nutr* 17: 159–167, 1998.

77. **Serón-Ferré M, Forcelledo ML, Torres-Farfan C, Valenzuela FJ, Rojas A, Vergara M, Rojas-Garcia PP, Recabarren MP, Valenzuela GJ**. Impact of chronodisruption during primate pregnancy on the maternal and newborn temperature rhythms. *PLoS ONE* 8: e57710, 2013.

78. **Seron-Ferre M, Reynolds H, Mendez NA, Mondaca M, Valenzuela F, Ebensperger R, Valenzuela GJ, Herrera EA, Llanos AJ, Torres-Farfan C**. Impact of maternal melatonin suppression on amount and functionality of brown adipose tissue (BAT) in the newborn sheep. *Front Endocrinol (Lausanne)* 5: 232, 2014.

79. **Shamsi NA, Salkeld MD, Rattanatray L, Voultsios A, Varcoe TJ, Boden MJ, Kennaway DJ**. Metabolic consequences of timed feeding in mice. *Physiol Behav* 128: 188–201, 2014.

80. **Shibata S, Moore RY**. Development of neuronal activity in the rat suprachiasmatic nucleus. *Brain Res* 431: 311–315, 1987.

81. **Shimomura H, Moriya T, Sudo M, Wakamatsu H, Akiyama M, Miyake Y, Shibata S**. Differential daily expression of*Per1* and*Per2* mRNA in the suprachiasmatic nucleus of fetal and early postnatal mice. *European Journal of Neuroscience* 13: 687–693, 2001.

82. **Silver R, LeSauter J, Tresco PA, Lehman MN**. A diffusible coupling signal from the transplanted suprachiasmatic nucleus controlling circadian locomotor rhythms. *Nature* 382: 810–813, 1996.

83. **Simonneaux V, Ribelayga C**. Generation of the melatonin endocrine message in mammals: a review of the complex regulation of melatonin synthesis by norepinephrine, peptides, and other pineal transmitters. *Pharmacol Rev* 55: 325–395, 2003.

84. **Sládek M, Jindráková Z, Bendová Z, Sumová A**. Postnatal ontogenesis of the circadian clock within the rat liver. *Am J Physiol Regul Integr Comp Physiol* 292: R1224–9, 2007.

85. **Sládek M, Sumová A, Kováciková Z, Bendová Z, Laurinová K, Illnerová H**. Insight into molecular core clock mechanism of embryonic and early postnatal rat suprachiasmatic nucleus. *Proc Natl Acad Sci U S A* 101: 6231–6236, 2004.

86. **Stark RI, Daniel SS**. Circadian rhythm of vasopressin levels in cerebrospinal fluid of the fetus: effect of continuous light. *Endocrinology* 124: 3095–3101, 1989.

87. **Stephan FK, Zucker I**. Circadian rhythms in drinking behavior and locomotor activity of rats are eliminated by hypothalamic lesions. *Proc Natl Acad Sci U S A* 69: 1583–1586, 1972.

88. **Swaab DF, Hofman MA, Honnebier MB**. Development of vasopressin neurons in the human suprachiasmatic nucleus in relation to birth. *Brain Res Dev Brain Res* 52: 289–293, 1990.

89. **Terazono H, Mutoh T, Yamaguchi S, Kobayashi M, Akiyama M, Udo R, Ohdo S, Okamura H, Shibata S**. Adrenergic regulation of clock gene expression in mouse liver. *Proc Natl Acad Sci U S A* 100: 6795–6800, 2003.

90. **Torrealba F, Parraguez VH, Reyes T, Valenzuela G, Serón-Ferré M**. Prenatal development of the retinohypothalamic pathway and the suprachiasmatic nucleus in the sheep. *J Comp Neurol* 338: 304–316, 1993.

91. **Torres-Farfan C, Mendez N, Abarzua-Catalan L, Vilches N, Valenzuela GJ, Seron-Ferre M**. A circadian clock entrained by melatonin is ticking in the rat fetal adrenal. *Endocrinology* 152: 1891–1900, 2011.

92. **Torres-Farfan C, Rocco V, Monsó C, Valenzuela FJ, Campino C, Germain A, Torrealba F, Valenzuela GJ, Seron-Ferre M**. Maternal melatonin effects on clock gene expression in a nonhuman primate fetus. *Endocrinology* 147: 4618–4626, 2006.

93. **Varcoe TJ, Boden MJ, Voultsios A, Salkeld MD, Rattanatray L, Kennaway DJ**. Characterisation of the maternal response to chronic phase shifts during gestation in the rat: implications for fetal metabolic programming. *PLoS ONE* 8: e53800, 2013.

94. **Varcoe TJ, Voultsios A, Gatford KL, Kennaway DJ**. The impact of prenatal circadian rhythm disruption on pregnancy outcomes and long-term metabolic health of mice progeny. *Chronobiol Int* 33: 1171–1181, 2016.

95. **Varcoe TJ, Wight N, Voultsios A, Salkeld MD, Kennaway DJ**. Chronic phase shifts of the photoperiod throughout pregnancy programs glucose intolerance and insulin resistance in the rat. *PLoS ONE* 6: e18504, 2011.

96. **Vermes I, Dohanics J, Tóth G, Pongrácz J**. Maturation of the circadian rhythm of the adrenocortical functions in human neonates and infants. *Horm Res* 12: 237–244, 1980.

97. **Vilches N, Spichiger C, Mendez N, Abarzua-Catalan L, Galdames HA, Hazlerigg DG, Richter HG, Torres-Farfan C**. Gestational chronodisruption impairs hippocampal expression of NMDA receptor subunits Grin1b/Grin3a and spatial memory in the adult offspring. *PLoS ONE* 9: e91313, 2014.

98. **Viswanathan N, Davis FC**. Maternal entrainment of tau mutant hamsters. *J Biol Rhythms* 7: 65–74, 1992.

99. **Vitaterna MH, King DP, Chang AM, Kornhauser JM, Lowrey PL, McDonald JD, Dove WF, Pinto LH, Turek FW, Takahashi JS**. Mutagenesis and mapping of a mouse gene, Clock, essential for circadian behavior. *Science* 264: 719–725, 1994.

100. **Voiculescu SE, Le Duc D, Roșca AE, Zeca V, Chiţimuș DM, Arsene AL, Drăgoi CM, Nicolae AC, Zăgrean L, Schöneberg T, Zăgrean A-M**. Behavioral and molecular effects of prenatal continuous light exposure in the adult rat. *Brain Res* 1650: 51–59, 2016.

101. **De Vries JI, Visser GH, Mulder EJ, Prechtl HF**. Diurnal and other variations in fetal movement and heart rate patterns at 20-22 weeks. *Early Hum Dev* 15: 333–348, 1987.

102. **Waddell BJ, Wharfe MD, Crew RC, Mark PJ**. A rhythmic placenta? Circadian variation, clock genes and placental function. *Placenta* 33: 533–539, 2012.

103. **Wharfe MD, Mark PJ, Waddell BJ**. Circadian variation in placental and hepatic clock genes in rat pregnancy. *Endocrinology* 152: 3552–3560, 2011.

104. **Wideman CH, Murphy HM**. Constant light induces alterations in melatonin levels, food intake, feed efficiency, visceral adiposity, and circadian rhythms in rats. *Nutr Neurosci* 12: 233–240, 2009.

105. **Williams LM, Martinoli MG, Titchener LT, Pelletier G**. The ontogeny of central melatonin binding sites in the rat. *Endocrinology* 128: 2083–2090, 1991.

106. **Zemdegs IZ, McMillen IC, Walker DW, Thorburn GD, Nowak R**. Diurnal rhythms in plasma melatonin concentrations in the fetal sheep and pregnant ewe during late gestation. *Endocrinology* 123: 284–289, 1988.

107. **Zhang P, Li G, Li H, Tan X, Cheng H-YM**. Environmental perturbation of the circadian clock during pregnancy leads to transgenerational mood disorder-like behaviors in mice. *Sci Rep* 7: 12641, 2017.

732 Figure legends

733 Figure 1

734 Current understanding of the key developmental milestones of the circadian timing system. Ontogeny
735 of the rat circadian system is well characterised. While SCN neurogenesis occurs prenatally in rats,
736 offspring are born relatively immature, with the majority of the circadian timing system developing
737 postnatally. Although less well characterised, SCN neurogenesis, synaptogenesis, RHT input and
738 cellular rhythmicity occur prenatally and at earlier stages of gestation in sheep and non-human primates
739 compared to rat. SCN neurogenesis (red vertical lines), RHT (yellow horizontal lines), and first evidence
740 for fetal SCN rhythmicity (black waveform).

741

742 Figure 2

743 Prenatal development in the absence of a molecular clock does not affect fetal growth or survival. Eight
744 week old *Bmal1* +/- mice were mated (n=9 litters) and dams killed at post coital day 17.5 by cervical
745 dislocation. Pups and placentas were rapidly dissected and weighed, with a fragment of pup tail
746 processed for genotype and sex using multiplex PCR. There was no effect of fetal genotype on number
747 of fetuses (A) fetal weight (B) or placental weight (C). *Bmal1* +/+ (closed columns), *Bmal1* +/- (shaded
748 columns), *Bmal1* -/- (open columns). Placentae from male fetuses were significantly heavier than those
749 of female fetuses, $P = 0.026$.

750 Figure 3

751 Model of Chronic Phase Shifts (CPS) used to induce maternal chronodisruption in a rat model, and the
752 impact on adult offspring. Pregnant dams were exposed to either a control photoperiod (A) or CPS (B)
753 throughout pregnancy and for one week after birth. At 12 months of age, offspring of CPS-exposed
754 dams (open bars and symbols) have increased plasma insulin (C) and plasma leptin (D) concentrations
755 compared to offspring of control dams (filled bars and symbols). Female offspring of CPS-exposed

dams also have increased adiposity (E) and poorer glucose tolerance (F) than female offspring of control dams. Data redrawn from (95).

Table 1. Commonly used techniques to induce circadian disruption in rodents and their impacts upon central and peripheral rhythmicity.

Method	**Technique**	**Impact on circadian rhythms (non-pregnant animals)**
Genetic manipulation	Mutation or knock out of clock genes	*ClockΔ19* mutant mice- Can entrain to light/dark cycle. Demonstrate free running period of 27 hours then onset of arrhythmicity when in constant darkness (99). Delayed onset and shorter duration of nocturnal melatonin secretion (39) Arrhythmic peripheral clock gene expression (36) *Bmal1* null mice- Partial entrainment to light/dark cycle. Complete behavioural arrhythmicity in constant darkness (10) Outcomes reported in mice strains deficient in melatonin, so effects of *Bmal1* knockout on melatonin secretion are unknown Arrhythmic peripheral clock gene expression (10)
SCN ablation	Surgical removal of SCN	Complete behavioural arrhythmicity (87) Constitutive melatonin secretion at 30% of control nocturnal peak concentrations (63). Constitutive peripheral clock gene expression (89)
Constant light	Animals housed in constant light	Free running behavioural rhythms or behavioural arrhythmicity (31, 60) Suppressed melatonin secretion (104) SCN neurons become desynchronised (60) Reduced amplitude and altered phase of peripheral clock gene expression (26)
Chronic phase shifts	Repeated changes to the timing of light exposure	Behavioural rhythms repeatedly shift to match the changing photoperiod (54, 93) Melatonin secretion shifts to match changing photoperiod (93) Corticosterone secretion arrhythmic (93) Reduced amplitude of peripheral clock gene expression (93)
Pinealectomy	Surgical removal of pineal gland	Normal rhythms of activity under light/dark or constant conditions (14, 68) Melatonin deficient (47) Minimal impact on SCN clock gene expression (1) Minimal impact on peripheral clock gene expression (22, 65)
Timed food access	Limit food availability to day time.	Disrupts normal patterns of activity such that animals are active during the day during periods of food availability. Phase shift in corticosterone secretion (79) No impact on SCN clock gene expression (28) Peripheral clock gene expression in metabolic tissues entrain to period of food availability or become de-regulated (28)

Table 2. Progeny outcomes following maternal circadian disruption.

Method	Species and exposure	Timing of exposure	Comparison group	Progeny outcomes after maternal chronodisruption
Genetic manipulation	Mouse – double mutant dams (*mPer2*^{Brdm1}/*Cry1*$^{-/-}$ or *mPer1*^{Brdm1}/*Per2*^{Brdm1}) lacking endogenous circadian rhythms mated to wild-type males to generate fetuses with functional circadian systems (32).	Throughout pregnancy and lactation, to weaning at 21 d of age. Dams and progeny housed in constant darkness throughout.	Progeny of heterozygous dams with functional *Per1*, *Cry1* and *Per2.*	Circadian rhythms: • All progeny displayed circadian rhythms in wheel-running activity, but 6 week-old progeny from mothers lacking functional circadian clocks had greater within-litter variability in timing of activity.
	Mouse – *ClockΔ19*+MEL females with disrupted circadian systems mated with wild-type males to generate heterozygote fetuses (93).	Throughout pregnancy and lactation, to weaning at 21 d of age. Dams and progeny housed in 12L:12D throughout.	Heterozygote progeny of crosses between wild-type females and *ClockΔ19*+MEL males.	Metabolic: • Adiposity (relative weight of summed epigonadal and retroperitoneal fat) normal in males and females at 3 and 12 months old. • Glucose tolerance by IPGTT: improved in young adult female progeny at 3 months of age (AUC glucose ↓25%), normal in young adult males and older adult (12 months old) male and female progeny. • Whole-body insulin sensitivity by IPITT: Unchanged glucose response to insulin in 3 and 12 month-old males and females.

SCN ablation	Sprague-Dawley rat - surgical ablation of maternal SCN (72).	SCN lesioned at day 7 of pregnancy (term 22 d). All groups lack light-entrainment of circadian rhythms from 2 d before birth onwards. In Experiment 4 only, pups cross-fostered between SCN-intact and SCN-lesioned groups by 24 h after birth.	Progeny gestated and reared by SCN-intact dams.	Circadian rhythms: • Loss of pineal gland N-acetyltransferase rhythms in 10 day-old mixed sex progeny. In cross-fostering study (Experiment 4), normal pineal NAT activity patterns exhibited by pups gestated by SCN-intact dams and reared by SCN-lesioned dams, variable patterns exhibited by pups gestated by SCN-lesioned dams and reared by SCN-intact dams, implying gestation as most critical period. • Rhythms of drinking behaviour under free-running conditions from weaning at 21 d until 42 d of age in male progeny had similar average cycle lengths but greater within-litter variability in timing of drinking in progeny gestated and reared by SCN-lesioned dams.
	Syrian hamster - surgical ablation of maternal SCN (18).	SCN lesioned at day 7 or 14 of pregnancy (term 16 d). All groups held in continuous dim light from the day after mating onwards.	Compared between progeny of dams ablated at different days of pregnancy and on basis of % of SCN remaining in dams.	Circadian rhythms: • Loss of within-litter synchrony in timing of wheel-running behaviour in mixed sex progeny recoded for 3-4 weeks after weaning in progeny of dams with SCN ablated at 7 d gestation, provided ~75% or greater of SCN was lost. Within-litter synchrony of behaviour maintained if SCN ablated at d 14 of gestation.
Manipulation of light exposure	Animals housed in constant light (99).	Wistar rats, continuous light exposure from days 12-21 of pregnancy. All dams and progeny housed in 12L:12D from d 21 of pregnancy onwards, weaning at 28 d of age.	Progeny of control rats housed in 12L:12D throughout study.	Circadian rhythms: • Decreased melatonin content of whole brains from neonatal (mixed sex) and adult male progeny. Neurobehavioral and cognitive: • Behavioral changes in adult male (<90 d old) progeny suggesting increased anxiety including decreased time in central area and increased defecations in open field test, less distance travelled and less time in open arms of elevated plus maze. • Evidence of impaired short-term memory in novel object recognition test (lower ratio of time spent with novel compared to familiar object) in adult male (<90 d old) progeny.

Animals housed in constant light (96).	Sprague-Dawley rats, continuous light exposure from day 10 of pregnancy until delivery. All dams and progeny housed in 12L:12D from delivery onwards, weaning at 21 d of age.	Progeny of control rats housed in 12L:12D throughout study.	Circadian rhythms: • Loss of day (1100 h)/night (2300 h) differences in plasma melatonin in adult males (90 days old). • Loss of day (1100 h)/night (2300 h) differences in hippocampal *Bmal1* gene expression, and altered day/night patterns of expression in several clock-controlled genes in adult males (90 days old). • Decreased day (1100 h)/night (2300 h) differences in plasma corticosterone in adult males (90 days old). Neurobehavioral and cognitive: • Memory by Morris Water Maze test: Impaired memory (longer time to find platform on days 2-4 of series of 5 days) in adult males (90 days old). Metabolic: • Glucose tolerance by IPGTT: ↑~60% fasting glucose and impaired glucose tolerance in adult males (90 days old).
Per1:GFP mouse - Mothers and progeny housed under short day (8L:16D), equinox (12L:12D) or long day (16L:8D) photoperiod (15).	Mating until weaning (21 d), then progeny either on same or different photoperiod for 28 days after weaning.	Between progeny of short day- and long day-housed dams.	Circadian rhythms: • Electrical and molecular rhythms in SCN of male progeny show longer period in progeny of short day compared to long day-housed dams. • Greater period of wheel-running cycle (behavioural period) under free-running conditions in progeny of short day compared to long day-housed dams (progeny sex not specified). • Greater circadian responses to variation in post-weaning photoperiod in animals exposed to short day photoperiod before weaning than in mice exposed to long day photoperiod before weaning (progeny sex not specified).
Animals exposed to dim light at night (4 lux) (6).	Swiss-Webster mice exposed from mating until birth, mating until weaning (21 d), or birth until weaning.	Progeny from dams exposed to 0 lux at night, from mating until weaning.	Neurobehavioral: • Increased anxiety like behaviors: Reduced time spent in open-arms and increased latency to enter arms in Elevated Plus Maze; increased latency to cross light chamber in Passive Avoidance Test; no change in Open Field Test. • No change in Forced Swim Test, Novel Object Recognition or Sucrose Anhedonia.

Chronic phase shifts	Repeated changes to the timing of light exposure (94).	Albino wistar rats, housed under CPS (lighting schedule reversal every 3-4 days) from morning after mating until 1 week after birth. Dams and progeny then held in 12L:12D for rest of study. Progeny weaned at 21 d of age.	Progeny of females held under 12L:12D throughout pregnancy and lactation.	Circadian rhythms: • Unchanged rhythms and levels of plasma glucose and insulin in both sexes at 3 months of age and of glucose in both sexes at 12 months of age. • Unchanged pattern but elevated circulating concentrations of insulin in female (↑83%) and male (↑110%) progeny and elevated plasma leptin in female (↑41%) and male (↑26%) progeny at 12 months of age. • Rhythms and light-induced changes in body temperature were normal in adult female and male progeny (35 weeks old). Neurobehavioral and cognitive: • Behavioural despair by forced swim test: Unchanged in 14 week-old male progeny. • Anxiety by open field test: Unchanged in 32 week-old male progeny. Metabolic: • Growth: Increased weight of female progeny from 40 weeks of age (15% heavier at 52 weeks old). Body weights of males normal. • Body composition: Increase in relative weight of epigonadal fat pad (↑29%) in 3 month-old males (not different in females or in either sex at 12 months old), and in relative weight of retroperitoneal fat pad (↑40%) in 12 month-old females (not different in males or in either sex at 3 months old). • Glucose tolerance by IPGTT: Unchanged at 3 months in both sexes. Impaired in females (AUC glucose ↑~20%) but unchanged in males at 12 months of age. • Insulin secretion by IPGTT: Unchanged at 3 months in both sexes. Impaired in males (AUC insulin ↓35%) but unchanged in females at 12 months of age. • Insulin sensitivity by IPITT: Unchanged at 3 months in both sexes. Impaired in females (AAC glucose ↓21%) but unchanged in males at 12 months of age.
	Repeated changes to the timing of light exposure (54).	Sprague-Dawley rats, housed under CPS (lighting schedule reversal every 3-4 days) from morning after mating until d 18 of pregnancy. Dams and progeny then held in 12L:12D for	Progeny of females held under 12L:12D throughout pregnancy.	Circadian rhythms: • Rhythms of SCN clock gene expression, locomotor activity and body temperature were normal in adult male progeny (80-90 days old). • Daily rhythm of plasma melatonin was absent (loss of variation and ~30% lower 24 h-average concentration) in adult male progeny (90 days old). • Daily rhythms of plasma aldosterone and corticosterone were absent, with increased average corticosterone concentrations in adult male progeny (90 days old). • Loss of daily fluctuation in corticosterone response to ACTH (controls have afternoon > morning response) in adult male progeny (90 days old).

		rest of study. Progeny weaned at 21 d of age.		• Decreased HR throughout daily cycle, with increased variation in HR during light and dark periods in adult male progeny (90 days old). • Increased BP at night and greater amplitude of BP rhythm, with increased variation in BP during light and dark periods in adult male progeny (90 days old). Metabolic: • Glucose tolerance by IPGTT: Impaired in males at 90 days of age.
	Repeated phase delays in the photoperiod (106).	Mice exposed to Chronic Circadian Disruption (CCD): 8 hour phase delays in the photoperiod every 2 days, from mating to day 18 gestation.	Progeny of dams exposed to 12L:12D throughout gestation.	Circadian rhythms: • Reduced amplitude of core-clock gene expression in SCN of F1 male and female offspring. Neurobehavioral: • Depressive-like behaviors: increased time spent immobile in the Forced Swim Test in female F1 progeny at postnatal day 28 and 56. Anti-depressive-like behaviour in male F1 progeny with decreased time spent immobile at postnatal day 28 and 56. Decreased time spent immobile in male and female F2 progeny. • Anhedonia- reduced sucrose preference in female F1 progeny at postnatal day 28, 56 and 84 and in male F1 progeny at postnatal day 28 and 84 only. Reduced sucrose preference in F2 female but not F2 male progeny.
Pinealectomy	Wistar rat - surgical removal of pineal gland before mating (24).	Absence of maternal melatonin throughout pregnancy and lactation (weaned at 21 d old).	Progeny of sham-operated dams.	Metabolic: • Normal fasting glucose in adolescent and young adult male and female progeny (4, 8, 16 and 18 weeks old). • Adiposity normal in males and females at 16 weeks old. • Glucose tolerance by IPGTT: normal in adolescent (8 week old) males, but impaired in 18 week-old males (AUC glucose ↑78%) and females (AUC glucose ↑77%). • Whole-body insulin sensitivity by IPITT: Unchanged glucose response to insulin in 18 week-old males and females. • Gluconeogenesis: Higher AUC glucose (measures GNG from pyruvate) during IPPTT in 18 week-old males (↑75%) and females (↑144%). Greater hepatic expression of PEPCK (rate limiting enzyme for GNG) in 18-week-old males (↑199%) and females (↑30%). • Insulin secretion: Similar basal but impaired insulin-stimulated insulin secretion in islets from 18 week-old males (females not assessed). • Insulin signalling pathways: Impaired insulin-stimulated activation of proximal signalling pathway in 18 week-old males and females (insulin-stimulated Akt phosphorylation ↓33% in males, ↓42% in females). Normal expression and

				insulin-stimulated activation of proteins in skeletal (soleus) muscle of 18 week-old males (females not assessed).
Timed food access	Wistar rat - limit food availability to light period (day time) (3).	Throughout whole of pregnancy and lactation (until weaning at 21 d old), or pregnancy only, or lactation only (by cross-fostering by 48 h after delivery).	Progeny of ad libitum-fed dams (continuous food access).	Metabolic: • Glucose tolerance by IPGTT: Exposure through gestation and lactation impaired glucose tolerance in 12 week-old male offspring (AUC glucose ↑40%). Gestation alone or lactation alone exposures did not alter glucose tolerance in males. No exposures altered glucose tolerance in females. • Whole-body insulin sensitivity by IPITT: Normal glucose response to insulin in 14 week-old males and females in all exposure groups. • Insulin secretion: Insulin secretion in islets from 16 week-old males was normal at basal glucose (5.6 mM) and under moderate glucose stimulation (8.3 and 11.1 mM) but insulin response to high glucose (16.7 mM) was reduced by 69% in male offspring exposed through gestation and lactation (progeny from gestation only or lactation only exposures not assessed, females not assessed).c