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NATURALLY OCCURRING PERINATAL GROWTH RESTRICTION PREVENTS THE DEVELOPMENT OF DIET INDUCED METABOLIC SYNDROME IN MICE.

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Background: Epidemiological studies show low birth weight increases the risk of developing metabolic syndrome (obesity, glucose intolerance and hypertension). Identification of an analogous model within an inbred mouse strain would facilitate mechanistic understanding. **Hypotheses:** 1) Perinatal growth restricted (PGR) mice have altered body composition, impaired glucose tolerance and hypertension (compared to isogenic controls). 2) The effects of PGR are amplified follow provision of a high fat diet. **Methods:** PGR C57Bl6 pups were identified at weaning (3wk) and paired with same sex normally grown littermates (n = 8 per group). Growth and feed intake was recorded weekly for 34 wk (17 wk on standard diet, then 17 wk on high fat diet). Glucose tolerance tests (GTT) and tail cuff systolic blood pressure (SBP) measurements were performed at the end of each diet period. **Results:** PGR pups weighed significantly less than controls throughout the study, despite increased caloric intake ($P<0.05$). PGR adults gained less than half as much weight as controls while on the high fat diet (HF). While on HF, glucose tolerance was significantly better in PGR mice than controls. Although SBP was significantly higher in PGR mice at 20 wk, following 17 wk of HF, SBP was lower in the PGR mice ($P<0.05$). **Conclusion:** Naturally occurring perinatal growth restriction is associated with (1) impaired postnatal weight gain; (2) increased SBP while on standard diet; and (3) decreased propensity towards diet-induced metabolic syndrome while on a high fat diet.

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RAPUNZEL, A NOVEL ZEBRAFISH MUTANT WITH LOSS OF NUTRITION-DEPENDENT GROWTH CONTROL

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Background and Purpose of Study: The essential genetics of normal human growth are not well understood. Size and form are maintained through the appropriate coordination of isometric and allometric growth. In addition, humans must be able to adapt their nascent developmental programs, such as growth, to ever changing environmental conditions, including nutritional status. When isometric or allometric control of growth is lost, disorders of growth control including overgrowth, undergrowth and cancer ensue. We use zebrafish and in particular, the zebrafish fin, as a genetically and physiologically tractable model to dissect the biology of growth control. Growth of the adult zebrafish caudal fin is episodic and isometric. In addition, when adult zebrafish are fasted, checkpoints rapidly abrogate caudal fin growth.

Methods and Summary of Results: An ENU-based forward mutagenesis screen identified the fin overgrowth mutant *rapunzel*. We used a positional cloning strategy to map the *rapunzel* mutation to a narrow critical region on zebrafish chromosome 16 containing 4 novel transcripts (wz750, wz17566, wz7309 and wz2605). 5′ and 3′ RACE were used to clone full-length cDNA's for these candidate genes, one of which contains a novel missense mutation. We are using *in situ* hybridization, zebrafish transgenesis, cell culture and morpholino knockdown to interrogate this genetic lesion further. Morphometric analysis reveals that fin growth in *rapunzel* is continuous and allometric, leading to overgrowth of all fins. Interestingly, when *rapunzel* mutants were fasted for 30 days, they added ~7 caudal fin ray segments (7.2 ± 2). This is in contrast to both wild type zebrafish (0 ± 1) and the *long fin* mutant (data not shown), both of which terminated fin ray growth rapidly upon fasting. Finally, we used blastula transplantation to explore the cell/tissue autonomy of the *rapunzel* mutation. Analysis of *rapunzel* chimeras revealed a critical role of the fin ray endothelium in generating overgrowth in *rapunzel* mutants.

Conclusions: Forward genetics identifies *rapunzel*, a fin overgrowth mutant wherein episodic and isometric control of fin growth is lost. Physiologically, *rapunzel* bypasses checkpoints that normally serve to abrogate growth when nutritional conditions are poor, moreover this property appears autonomous to the fin ray endothelium. Positional cloning has mapped *rapunzel* to a narrow critical region containing 4 novel transcripts. Progress on cloning the *rapunzel* mutation will be presented.

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ENHANCING THE EXPRESSION OF THE DNA REPAIR/REDOX ENZYME, APE1/REF-1, REDUCES NEUROTOXICITY INDUCED BY IONIZING RADIATION: IMPLICATIONS FOR DECREASING PEDIATRIC NEUROCOGNITIVE DYSFUNCTION.

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Neurotoxicity which includes both neurocognitive dysfunction commonly called "chemobrain" and peripheral neuropathy, occurs frequently in pediatric patients who receive ionizing radiation (IR) for the treatment of cancer. Despite the prevalence of these side effects, little work has been done to elucidate the mechanisms of or interventions to prevent neurotoxicity. Apurinic/apyrimidinic endonuclease/redox effector factor (Ape1) repairs AP sites in DNA secondary to oxidative damage and regulates the redox state of transcription factors. Because IR produces reactive oxygen species and DNA damage, we examined whether overexpressing Ape1 in primary neuronal cultures reduces toxicity after IR. To decrease the expression of Ape1, rat hippocampal or sensory neuronal cultures were exposed to small interfering RNA to Ape1 (Ape1siRNA) or to scramble siRNA (SCsiRNA) as a control. To overexpress Ape1, cells treated with siRNA were subsequently exposed to adenoviral constructs containing Ape1 or to a vector control. Cultured cells in the absence or presence of altered expression of Ape1 were then exposed to IR and three endpoints examined, cell viability, DNA double strand breaks, and neurotransmitter release. Exposing neurons to increasing amounts of IR resulted in a dose–dependent increase in cell death 24 hours after the treatment. Reducing Ape1 expression with Ape1siRNA by ~ 85-95% significantly augmented IR-induced cell death compared to cultures treated with SCsiRNA. In a similar manner, reductions in Ape1 in neuronal cultures doubled the IR-induced phosphorylation of histone 2A.X, indicating an increase in DNA double-strand breaks. Overexpressing Ape1 in neuronal cultures treated with SCsiRNA or Ape1siRNA resulted in a significant increase in cell viability and a 4-6 fold reduction in H2A.X phosphorylation after exposure to IR. IR significantly reduced the stimulated release if calcitonin gene-related peptide (CGRP) from sensory neurons from 10.2 ± 0.5 5 of the total content of CGRP to 7.5 0.7%. This reduction in release was augmented by reducing Ape1 expression by 85% and revered by overexpressing Ape1. These data demonstrate that Ape1 plays an important role in minimizing neurotoxicity secondary to oxidative stress and modulation of Ape1's functions could lead to protection of neuronal cell damage and killing during IR treatments.

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WHAT MAKES AN ADOLESCENT SEXUALLY ACTIVE? INTRA- AND INTERPERSONAL INFLUENCES.

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Purpose: Sexual abstinence, a healthy people 2010 goal, is a period of time in which a person does not have sex. We examined intra- and interpersonal factors associated with the decision to have sex after a period of abstinence among young women at high STD risk. **Methods**: With IRB approval, we recruited 378 women (ages 14 – 17 at enrollment, >90% African American) from clinics in high STD risk communities. Subjects (ss) completed face-to-face interviews every 3 months, and daily diaries for 3 month periods twice a year. We PCR tested self-collected vaginal swabs for *C. Trachomatis, N. Gonorrhea*, and *T. Vaginalis* every 3 months, and weekly swabs during diary collection. On daily diaries, ss provided partner-specific coital behaviors. We defined an abstinence "run" (consecutive days of no sex) as our unit of analysis. Runs ended with a diary report of sex, and were censored with a missing diary day or the start or end of diary collection. We used frailty models to estimate the effect of intrapersonal factors, interpersonal factors, and STI diagnosis on time to ending a run. Frailty models are adaptations of proportional hazards models that control for multiple observations (in this case "runs" of abstinence) from one individual. Intrapersonal factors included daily positive and negative mood (3 items each, range 3-15, α=0.81 and 0.76 respectively), and daily sexual interest (1 item, range 1-5). Partner-specific interpersonal factors included daily partner support (4 items, range 0-4, α=0.85), and overall relationship quality (6 items, range 6-24, α=0.91). Recent STIs were diagnosed in the quarterly (or 3 month) interview immediately before the run. **Results**: 378 ss contributed 6070 runs of abstinence, with 55.3% ending with sex and 44.7% censored. The median length of an abstinence run was 10 days (95% CI 9, 11 days) and mean 39.1 days (std error = 0.71). Each year increase in age increased the hazard of ending a run by 22% ($p<.001$). Each unit increase in sexual interest increased the hazard of ending a run by 22% ($p<.001$); each unit increase in positive mood increased the hazard by 2% ($p<.001$); negative mood was not a significant predictor. Each unit increase in partner support increased the hazard of ending a run by 25% ($p<.001$); and each unit increase in relationship quality increased the hazard by 5% ($p<.001$). A recent STI decreased the hazard of ending a run by 17% ($p<0.01$). **Conclusions**: The decision to have sex after a period of abstinence was strongly influenced by relationship characteristics as well as sexual interest and mood. This challenges popular notions of "casual" adolescent sex (e.g. "friends with benefits"). Adolescent STD prevention may be enhanced by tailoring counseling to these intra- and interpersonal circumstances.

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CUMULATIVE HYPERCAPNIA AND RISK OF INTRAVENTRICULAR HEMORRHAGE AMONG VERY LOW BIRTH WEIGHT INFANTS

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BACKGROUND: Intraventricular hemorrhage (IVH) remains a concern among very low birth weight infants (VLBWI). Several factors have been incriminated in the development of IVH. Although hypercapnia early in life has been associated with an increased risk of IVH, the effect of cumulative exposure to hypercapnia (CEH) on the risk and severity of IVH has not been explored. **OBJECTIVE:** To determine whether CEH in the first week of life is associated with risk and/or severity of IVH in VLBWI**. METHODS:** Infants with birth weight ≤ 1250g and survived for >7 days were included. Blood gas measurements during the first week of life and known risk factors for IVH were obtained from medical records. CEH was calculated as the sum of hours of infants' exposure to $pCO_2 > 45$ mmHg. Levels of CEH were compared between infant groups (group 1: no or grade 1 IVH; group 2: grade 2-4 IVH) using t-test. A stepwise logistic regression (LR) analysis was used to adjust for confounding variables and to assess the effect of CEH on IVH. **RESULTS:** A total of 103 VLBWI were included. Infants in group 2 were more likely to be male, had lower gestational age, illicit drug exposure *in utero*, maternal $MgSO_4$, delivery room resuscitation, hypotension, pulmonary hemorrhage, pneumothorax, and higher mean levels of CEH at 3 and 7 days of life ($p<0.05$). There was a significant increase in the occurrence of IVH with increasing quartiles of CEH (Pearson correlation $p<0.05$), as well as a direct and positive correlation between severity of IVH and increasing quartiles of CEH (Kendall's tau-b correlation, $p=0.025$). Male gender (OR=3.3, 95% CI: 1.3 – 8.4, $p=0.01$), pneumothorax (OR=10.2, 95% CI: 1.9 – 55.2, $p=0.007$), and CEH in the first week of life (OR=1.8, 95% CI: 1.2 – 2.8, $p=0.006$) significantly increased the risk for developing IVH. **CONCLUSIONS:** Cumulative exposure to hypercapnia during the first week of life is associated with increased risk and severity of IVH.

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CORTISOL VALUES DO NOT CORRELATE WITH BLOOD PRESSURE IN PRETERM NEONATES.

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Purpose: Despite scant evidence describing the role of cortisol in blood pressure (BP) regulation in the neonatal population, hydrocortisone is frequently used to treat preterm infants with hypotension resistant to volume and pressor agents. Circulating cortisol exists in 2 forms: protein bound (90%) and the biologically active free form (10%). No prospective studies have examined the relationship between total cortisol and free cortisol in the preterm neonate during the first postnatal week, or the relationship between cortisol values and BP in this group of infants. The primary purpose of this study, therefore, is to characterize total and free cortisol levels over the first week of life and examine their relationship to BP values in the preterm neonate. **Methods**: All infants with an estimated gestational age (EGA) ≤32 weeks were eligible for enrollment. After obtaining parental consent, blood samples were obtained 4 times during the first postnatal week (e.g. 24-36hr, 48-72hr, DOL 5 and DOL 7). Data including BP, respiratory status and fluid balance were also obtained at these times. Total and free cortisol was measured using HPLC mass spectrometry and radioimmunoassay. **Results**: 35 infants of EGA 30-32 weeks were enrolled between May 2005 and January 2006. Total cortisol values ranged from 1-35μg/dl (7.6 ± 8.5[s.d]) at 24-36hr and trended down to 1-14μg/dl (4.0 ± 3.5) by DOL 7. Free cortisol values ranged from 0.02-25μg/dl (2.2 ± 5.6) at 24-36hr and also showed a downward trend by DOL 7 to 0.02-0.86μg/dl (0.17 ± 0.2). The ratio of free to total cortisol varied from 2-74% (11.8% ± 17%) at 24-36hr to 1-10% (3.9% ± 1.9%). Although there was a significant linear increase in BP over the first week (mean BP at 24-36hr, 41 ± 6.2 vs. DOL 7, 49 ± 7.6 $p<.001$), there was no correlation between BP and total cortisol or free cortisol at 24-36hr, 48-72hr or DOL 7 ($r = -0.05$, -0.04 and -0.1 (total cortisol); -0.01, 0.06, and -0.02 (free cortisol) respectively). A moderate correlation ($r = 0.45$) between BP and total and free cortisol was noted on DOL 5. **Conclusions**: Total cortisol, free cortisol and the percentage of free cortisol trend downward in preterm neonates with an EGA of 30-32 weeks during the first week of life, though a larger sample size will be required to detect if this trend is significant. There is no correlation between total or free cortisol with BP during this time period, making the utility of a random cortisol value to guide treatment for hypotension questionable. Whether this is also true for infants of younger gestational ages is unknown; however, we are in the process of evaluating a concurrent cohort of infants with EGA 24-29 weeks to further examine this question.