

Periconceptional alcohol alters adult renal and cardiac function in a sex dependent manner

E.S. Dorey,¹ E.M. Gardebjer,¹ F. Campbell,¹ T.M. Paravicini,¹ K.A. Weir,¹ M.E. Wlodek² and K.M. Moritz,¹ ¹The University of Queensland, St Lucia, QLD 4072, Australia and ²The University of Melbourne, Melbourne, VIC 3010, Australia.

The periconceptional period is becoming widely recognised as a critical window for programming of adult onset disease. During the first month of gestation, 22.5% of women report consuming alcohol (Ethen *et al.*, 2009), with the ramifications for offspring currently unknown. Alcohol exposure throughout gestation and undernutrition during the periconceptional period have both been reported to cause decreased kidney weight, altered nephron endowment and increased blood pressure in offspring (Watkins *et al.*, 2008; Gray *et al.*, 2010). The effects of periconceptional alcohol exposure on pathophysiological changes in the adult have not been examined.

To investigate the effects of periconceptional alcohol exposure (PCEtOH), female Sprague Dawley rats were placed on a liquid control diet or a liquid 12.5% v/v ethanol diet from 4 days before mating until embryonic day 4. At 6, 12 and 19 months, offspring were placed in metabolic cages for 24 hours for collection of urine and analysis of food and water intake. A dehydration challenge was performed at 6 and 19 months to assess urine concentrating ability. At 19 months, normal water intake was measured over 3 days with rats in their home cages. Urinary excretion of Na⁺, K⁺ and Cl⁻ was examined at all ages and in both conditions. The osmolality of the urine, urinary albumin excretion and urinary creatinine concentration were calculated at 19 months. Echocardiography was performed at 12 months under general anaesthesia (2% isoflurane). Males and females were analysed separately.

PCEtOH exposure increased urine flow corrected for body weight in male offspring at both 6 and 12 months of age ($P<0.05$). In female offspring, PCEtOH exposure did not affect corrected urine flow at 6 months, but increased urine flow at 12 and 19 months ($P<0.05$). Home cage water intake was increased in female offspring at 19 months ($P<0.05$). Urine osmolality, electrolyte, albumin and creatinine excretion were unchanged in either sex at any age. Under dehydration challenge, all PCEtOH offspring concentrated urine appropriately at 6 months. Female PCEtOH offspring exhibited an increased flow rate during dehydration when compared to controls ($P<0.05$) at 19 months, with no change in urine osmolality. Echocardiography studies showed that in PCEtOH female offspring, there was a decrease in cardiac output (CO, $P<0.05$), an increase in left ventricular internal diameter during systole ($P=0.05$) and a tendency for fractional shortening to be decreased ($P=0.08$).

This study demonstrates that PCEtOH can influence renal and cardiac function in offspring in an age and sex dependent manner. Male offspring exhibit alterations to renal function beginning at 6 months, whilst female offspring appear protected from early renal dysfunction. By 12 months, females have an increased urinary flow rate that persists under challenge at 19 months. These results indicate possible changes to the kidney at the tubular level, or alterations to hormonally mediated water homeostasis. More investigations are needed to understand the mechanisms underlying the mild diuresis. Changes to cardiac function in females indicate alterations in contractility of the heart with an associated decrease in CO, which appears independent of a hypovolemic state, indicated by an increase in water intake under normal conditions. Our study is the first to establish that PCEtOH can result in functional alterations to the heart and kidneys that develop with age differently between the sexes. Our novel results suggest that women planning to become pregnant should avoid consumption of ethanol.

- Ethen M, Ramadhani T, Scheuerle A, Canfield M, Wyszynski D, Druschel C & Romitti P (2009). Alcohol Consumption by Women Before and During Pregnancy. *Maternal and Child Health Journal* **13**, 274-285.
- Gray SP, Denton KM, Cullen-McEwen L, Bertram JF & Moritz KM (2010). Prenatal Exposure to Alcohol Reduces Nephron Number and Raises Blood Pressure in Progeny. *Journal of the American Society of Nephrology* **21**, 1891-1902.
- Watkins AJ, Wilkins A, Cunningham C, Perry VH, Seet MJ, Osmond C, Eckert JJ, Torrens C, Cagampang FRA, Cleal J, Gray WP, Hanson MA & Fleming TP (2008). Low protein diet fed exclusively during mouse oocyte maturation leads to behavioural and cardiovascular abnormalities in offspring. *Journal of Physiology London* **586**, 2231-2244.