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MATERNAL EXERCISE DURING PREGNANCY
AFFECTS THE RAT MUSCULOSKELETAL SYSTEM
AND INDICES OF ENERGY METABOLISM

A thesis presented in partial fulfilment
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ABSTRACT

The developmental origins of health and disease hypothesis postulates that environmental cues perceived by the developing organism during early life program long-term health outcomes. A series of studies were undertaken to examine the developmental programming effects of maternal exercise during pregnancy on offspring musculoskeletal health and energy metabolism using a rat model. Firstly, an exercise that did not cause a potentially confounding stress response in the exercising animal was identified. Secondly, pregnant dams then performed this exercise and its effects on fetal growth and the maternal stress response were quantified. Finally, the offspring of dams that exercised throughout pregnancy were allowed to grow to maturity, and the effects of maternal exercise on their musculoskeletal health and energy metabolism were assessed. Throughout these experiments, body composition was assessed by dual-energy X-ray absorptiometry, and tibial parameters were measured using peripheral quantitative computed tomography. Maternal stress was quantified by measurement of faecal corticoid metabolites. Serum concentrations of the fully and undercarboxylated forms of the bone-derived hormone osteocalcin, and expression of genes related to osteocalcin carboxylation, were measured to explore their role in the response of offspring bone and energy metabolism to maternal exercise.

Two exercise types, rising to an erect bipedal stance and tower climbing, were initially tested in non-pregnant rats. Both rapidly caused changes in the tibias of exercised animals without inducing stress. In pregnant rats, both exercises increased fetal growth relative to controls, and neither caused a physiological stress response in the dams. Since rising to an erect bipedal stance had the greater effect on fetal growth, it was selected for use in the final study in which the offspring were grown to maturity. Maternal exercise throughout pregnancy was associated with sex-dependent changes in

the bone and body composition of the mature offspring. Male offspring of exercised dams had increased adiposity and serum undercarboxylated osteocalcin concentrations, while offspring of both genders had lower volumetric bone mineral density at the tibial diaphysis, relative to controls. These results suggest that maternal exercise has long-term effects on the musculoskeletal system and energy metabolism, and that undercarboxylated osteocalcin may play a role in these effects.

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LIST OF PUBLICATIONS

Rosa BV, Firth EC, Blair HT, Vickers MH, Morel PCH, Cockrem JF (2010) Short-term voluntary exercise in the rat causes bone modeling without initiating a physiological stress response. *Am J Physiol Regul Integr Comp Physiol* 299: R1037-R1043.

Rosa BV, Firth EC, Blair HT, Vickers MH, Morel PCH (2011) Voluntary exercise in pregnant rats positively influences fetal growth without initiating a maternal physiological stress response. *Am J Physiol Regul Integr Comp Physiol* 300: R1134-R1141.

Rosa BV, Blair HT, Vickers MH, Morel PCH, Cockrem JF, Firth EC (2012) Voluntary exercise in pregnant rats improves post-lactation maternal bone parameters but does not affect offspring outcomes in early life. *J Musculoskelet Neuronal Interact* 12: 199-208

Rosa BV, Blair HT, Vickers MH, Knight CG, Morel PCH, et al. (2013) Serum concentrations of fully and undercarboxylated osteocalcin do not vary between estrous cycle stages in Sprague-Dawley rats. *Endocrine* 44: 809-811.

Rosa BV, Blair HT, Vickers MH, Dittmer KE, Morel PCH, Knight CG, Firth EC (2013) Moderate exercise during pregnancy in Wistar rats alters bone and body composition of the adult offspring in a sex-dependent manner. *PLOS ONE* 8: e82378.

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Brielle V. Rosa, Elwyn C. Firth, Hugh T. Blair, Mark H. Vickers, and Patrick C. H. Morel. *American Journal of Physiology: Regulatory Integrative and Comparative Physiology* (2011) 300: R1134-1141.

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