**Progress Report for Endocrine Society of Australia Research Higher Degree Scholarship**

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**Name**: Tian Nie

**Institution**: Department of Medicine, Austin Hospital, The University of Melbourne, VIC, Australia

**Project title**: The effects of estradiol administered during puberty and in adulthood in a transgender male-to-female mouse model on bone cell metabolism, structure, and strength.

**Supervisors**: Associate Professor Rachel Davey, Professor Mathis Grossmann, Professor Jeffrey Zajac, Dr. Varun Venkatesh.

**Background**: Despite the estimate of 150,000 to 300,000 transgender individuals in Australia1 and rapidly rising demand, transgender health remains a neglected area. Transgender girls and women who were assigned male at birth may choose to be treated with estradiol as part of their gender affirming hormone therapy (GAHT) to alleviate their gender dysphoria. For transgender adolescents, they first undergo puberty suppression with gonadotrophin-releasing hormone agonists (GnRHa) in early puberty to avoid development of undesired pubertal characteristics of their natal sex, prior to administration of sex hormone of the desired gender in late puberty. Post-pubertal transgender women do not require puberty blocking, and may commence GAHT after consulting with medical professionals. GAHT consists of treating trans women with estrogens alone or in combination with anti-androgens. As sex steroids modulate physiological processes in wide range of tissues such as the gonads, muscle and bone, GAHT is likely to have long-term effects on the health of transgender individuals.

Bone is a dynamic organ, which is constantly remodelled to ensure renewal of the skeleton and structural integrity. In adolescents, the sex steroids estradiol and testosterone play a crucial role in bone growth and the attainment of peak bone mass. In adults, sex steroids are critical for maintaining bone mass and strength. Given their profound effects on bone, it is likely that puberty suppression and treatment with sex hormones during GAHT in transgender individuals will cause permanent, detrimental effects on bone.

Following GAHT in humans of both sexes, the major determinant of bone integrity is likely to be estradiol2. However, transgender women given estradiol therapy tend to have low bone mineral density and higher fracture risk after treatment3,4, and the full effects of GAHT on bone is unknown. Both estradiol and testosterone circulate and estradiol is also produced locally in bone from the aromatasation of testosterone by osteoblasts, the bone forming cells. There are two major unanswered questions in bone biology: How do the circulating levels of estradiol and testosterone determine the local bone concentrations of these sex steroids? and 2) What are the effects of gender affirming hormone therapy on bone cell metabolism, structure, and strength when administered during adolescence or in adulthood?

These questions are of biological importance in understanding sex steroid action in bone and also have major therapeutic implications on the skeletal health of transgender individuals. We will use pre-clinical mouse models to precisely mirror transgender individuals undergoing GAHT at puberty and in adulthood. A key advantage of mice is that they will provide essential information relating to the testosterone and estradiol concentrations within bone as well as bone cell activity microstructure, density and breaking strength using analyses that cannot be performed in humans in the absence of an invasive bone biopsy. Despite the differences between mice and humans, their genetic and pathophysiological similarities allow to advance our understanding of physiology and pathophysiology5. Clinically relevant findings can be extrapolated from mouse-specific findings if the differences are fully appreciated.

**Hypothesis**:The local concentrations of estradiol within bone in males is primarily derived by the aromatisation of testosterone by osteoblasts and not from circulating concentrations of estradiol. The aromatase-derived estradiol then acts locally on surrounding bone cells to maintain trabecular and cortical bone volume and bone strength.

**Specific Hypothesis 1:** Exogenous estradiol treatment in castrated male-to-female prepubertal mice will not be sufficient to restore cortical and trabecular bone volume or bone strength to levels observed in male sham controls.

**Prepubertal** male mice that are castrated at 5 weeks of age will have decreased bone mineral density (BMD) and size compared to male controls. Treatment with a physiologically female dose of estradiol via silastic implant 3 weeks post-surgery for a period of 12 weeks will not be sufficient to restore bone size to that observed in male sham controls as periosteal apposition is stimulated by testosterone which remains decreased in these mice. Since I hypothesise that the levels of estradiol within bone in males is primarily derived by the aromatization of testosterone by osteoblasts and not from circulating estradiol, the levels of estradiol within bone will remain low following estradiol treatment and will not be sufficient to increase cortical and trabecular bone density or bone strength to the levels observed in male sham controls.

**Specific Hypothesis 2:** **Adult** male mice treated with estradiol via silastic implant for 12 weeks will exhibit increased serum estradiol and decreased serum testosterone levels relative to male controls, due to negative feedback of estradiol on the hypothalamic–pituitary–gonadal (HPG) axis6. The decrease in serum testosterone levels will result in a concomitant decrease of testosterone levels within bone and subsequently decreased estradiol levels within bone, derived from the aromatisation of testosterone by osteoblasts. The decreased estradiol levels within bone will result in increase bone resorption, decrease cortical and trabecular bone volume and decreased bone strength compared with sham male controls.

**Overall Aim**: To determine the effects of GAHT in prepubertal and adult mice on bone cell metabolism, microstructure and breaking strength and correlate this with the serum and local bone concentrations of testosterone and estradiol.

**Aim 1:** To evaluate the physiological changes in serum and bone concentrations of estradiol and testosterone in prepubertal male-to-female mice and correlate these hormonal changes with bone cell metabolism, bone microstructure and bone breaking strength.

In humans, transitioning in adolescence first involves arresting puberty with hormone therapy starting at a later age. To mimic the arrest of puberty, male mice were orchidectomised (Orx) prior to puberty at 5 weeks of age and were administered either a physiological dose of estradiol (previously determined in a pilot conducted in 2020) via silastic implant or an empty vehicle silastic implant at 3 weeks post-gonadectomy for a period of 12 weeks. Controls included vehicle treated intact sham surgery males, sham surgery females, and Orx males, n=9-10/group.

All procedures were conducted in accordance with the ARRIVE guidelines7. To enable dynamic histomorphometric analysis of bone formation, all mice received two intraperitoneal injections of 20mg/kg body weight calcein at 10 and 3 days prior to sacrifice. Analyses were performed in a blinded fashion.

**Progress in 2021**: All experiments were completed in mid 2021, with data analysis ongoing. Serum estradiol measured by LC-MS/MS analysis was higher in Orx males treated with estradiol compared to vehicle treated intact males, females, and Orx males (*P*<0.0001). These analyses were performed in collaboration with Professor David Handelsman and Reena Desai at the ANZAC Research Institute.

Bone microstructure was determined using microCT. Orx pubertal males had decreased periosteal circumference (*P*<0.0001) and cortical thickness (Ct.Th) (*P*<0.001) compared to intact male controls. Periosteal circumference was increased in estradiol-treated Orx males compared to Orx controls and intact females but remained lower compared to intact males (*P*<0.0001). This is consistent with the periosteal apposition of bone during puberty in males requiring androgen action. Ct.Th was increased in estradiol-treated Orx males by approximately 50% compared to vehicle treated intact males, females, and Orx males (*P*<0.0001). In trabecular bone, Orx pubertal males had decreased trabecular bone volume (BV/TV), thickness (Tb.Th) and number (Tb.N) (*P*<0.05) compared to intact male controls. BV/TV was increased in Orx males following estradiol treatment, which was associated an increase in both TbN and TbTh (*P*<0.0001).

Mechanical testing conducted in collaboration with Associate Professor Kathryn Stok has been completed with data analysis ongoing. Resin-embedded femurs are currently being sectioned for dynamic histomorphometry, and bone samples are being processed for estradiol and testosterone measurements via LC-MS/MS analysis.

**Preliminary analysis for this aim suggest that exogenous estradiol treatment in a prepubertal mouse model of male-to-female transition protects against orchidectomy-induced bone loss.**

**Aim 2:**  To assess the effects of GAHT in adult male-to-female mice on bone cell metabolism, bone microstructure and breaking strength, and to correlate this with the serum and bone concentrations of testosterone and estradiol**.**

Transitioning in human adults does not require gonadectomy. To mimic transitioning in adulthood, male mice were administered either a physiological dose of estradiol (previously determined in 2020) via silastic implant or an empty vehicle implant at 16 weeks, a time at which peak bone mass had been achieved. Tissues were collected 12 weeks post-treatment. Controls included vehicle treated intact males and females, n=9-10/group.

All procedures were conducted in accordance with the ARRIVE guidelines7. To enable dynamic histomorphometric analysis of bone formation, all mice received two intraperitoneal injections of 20mg/kg body weight calcein at 10 and 3 days prior to sacrifice. Analyses were performed in a blinded fashion.

**Progress in 2021**: All experiments were completed in late 2021, with data analysis ongoing. Preliminary LC-MS/MS analysis showed that serum estradiol was higher in males treated with estradiol compared to female and male controls (*P*<0.0001). Serum testosterone in males treated with estradiol was decreased compare to male controls, consistent with negative feedback on the HPG axis from the estradiol treatment. These analyses were conducted in collaboration with Professor David Handelsman and Reena Desai at the ANZAC Research Institute.

Estradiol in male mice increased cortical thickness which was accompanied by a decrease in medullary volume (*P*<0.05), consistent with the actions of estradiol to stimulate the endocortical deposition of bone. In contrast, periosteal circumference was unaffected in males treated with estradiol. Estradiol treatment increased trabecular BV/TV compared to male and female controls due to an increase in Tb.N (*P*<0.0001) while Tb.Th was unaffected.

Preliminary bone strength data from mechanical testing conducted in collaboration with A/Prof Kathryn Stok suggest that estradiol treatment in male mice increases bone stiffness, thereby increasing the maximum force a bone can withstand prior to fracture. Further data analysis is ongoing.

Femurs are currently being sectioned for dynamic histomorphometry, and bone samples are being processed for estradiol and testosterone measurements via LC-MS/MS analysis.

**Preliminary data suggests that exogenous estradiol treatment in an adult pre-clinical model of male-to-female transition increases both cortical and trabecular bone volume, leading to an increase in bone strength.**

**Abstracts and Oral Presentations**

* 2021 Virtual ECTS and GEMSTONE COST-Action Digital Masterclass for Ph.D. Students, Trainees, and Young Investigators.
* **Nie T**, Venkatesh V, Golub S, Zajac JD, Grossmann M, Davey RA. *Estradiol preserves endocortical bone deposition in an adolescent pre-clinical model of gender affirming hormone therapy.* Austin Hospital ResearchFest 2021, Abstract # 54783.
* **Nie T**, Venkatesh V, Golub S, Zajac JD, Grossmann M, Davey RA. *Estradiol preserves endocortical and trabecular bone in an adolescent male-to-female mouse model of gender affirming hormone therapy*. ESA-SRB-ANZBMS ASM 2021, Abstract #144, ANZBMS Christopher and Margie Nordin Young Investigator Award Finalists session.

**Awards**

* 2021 ASBMR Young Investigator Awards for Ph.D. Training.
* 2021 Austin LifeSciences Prize for Discovery Research.
* AusBiotech 2021 AbbVie Student Scholarship.

**Manuscript in preparation (first author):**

* **Nie T**, Venkatesh V, Grossmann M, Zajac JD, Davey RA. The utility of preclinical rodent models in understanding the bone health of transgender individuals undergoing gender affirming hormone therapy.

**Publications during scholarship period:**

Grossmann, M., Fui, M. N. T., **Nie, T**., Hoermann, R., Clarke, M. V., Cheung, A. S., … Davey, R. A. (2021). Changes in white adipose tissue gene expression in a randomized control trial of dieting obese men with lowered serum testosterone alone or in combination with testosterone treatment. *Endocrine*. <https://doi.org/10.1007/s12020-021-02722-0>

**References**

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