Bone 74 (2015) 191–198

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Original Full Length Article

Longitudinal bone mineral content and density in Rett syndrome and their contributing factors☆

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ARTICLE INFO

Article history:
Received 19 September 2014
Revised 24 December 2014
Accepted 30 January 2015
Available online 7 February 2015

Edited by: Nuria Guanabens

Keywords:
Rett syndrome
Densitometry
Bone density
Bone mass
Fracture

ABSTRACT

Bone mass and density are low in females with Rett syndrome. This study used Dual energy x-ray absorptiometry to measure annual changes in z-scores for areal bone mineral density (aBMD) and bone mineral content (BMC) in the lumbar spine and total body in an Australian Rett syndrome cohort at baseline and then after three to four years. Bone mineral apparent density (BMAD) was calculated in the lumbar spine. Annual changes in lean tissue mass (LTM) and bone area (BA) were also assessed. The effects of age, genotype, mobility, menstrual status and epilepsy diagnosis on these parameters were also investigated.

The baseline sample included 97 individuals who were representative of the total live Australian Rett syndrome population under 30 years in 2005 (n = 274). Of these 74 had a follow-up scan. Less than a quarter of females were able to walk on their own at follow-up. Bone area and LTM z-scores declined over the time between the baseline and follow-up scans. Mean height-standardised z-scores for the bone outcomes were obtained from multiple regression models. The lumbar spine showed a positive mean annual BMAD z-score change (0.08) and a marginal decrease in aBMD (−0.04). The mean z-score change per annum for those 'who could walk unaided' was more positive for LS BMAD ($p = 0.040$). Total body BMD mean annual z-score change from baseline to follow-up was negative (−0.03). However this change was positive in those who had achieved menses prior to the study (0.03, $p = 0{,}040$). Total body BMC showed the most negative change (−0.60), representing a decrease in bone mineral content over time. This normalised to a z-score change of 0.21 once adjusted for the reduced lean tissue mass mean z-score change (−0.21) and bone area mean z-score change (−0.14).

Overall, the bone mineral content, bone mineral density, bone area and lean tissue mass z-scores for all outcome measures declined, with the TB BMC showing significant decreases.

Weight, height and muscle mass appear to have impacts on bone formation and we recommend that nutritional intake should be closely monitored and a physical activity plan developed to optimise bone health. Pubertal progression should also be assessed in conjunction with serial densitometry assessments to track bone mass and density changes over time.

Introduction

Rett syndrome (RTT) a rare genetic disorder mainly affecting females, results in marked impairments in the development of neurological function including communication, gross motor function and hand use [1,2]. The disability is complicated by the development of comorbidities such as scoliosis [3], epilepsy [4], impaired growth [5], autonomic disturbances [6], decreased bone density and skeletal abnormalities [7–11]. More than 200 mutations in the *MECP2* gene have been shown to be the main cause of RTT, however eight occur more commonly [13–15]. The wide range of phenotypic severity observed can be explained in part by the type of mutation [1,2] and the degree of skewing of X-chromosome inactivation [16].

Abbreviations: RTT, Rett syndrome; TB, total body; LS, lumbar spine; DXA, densitometry; aBMD, areal bone mineral density; BMC, bone mineral content; BMAD, volumetric bone mineral density; LTM, lean tissue mass; BA, bone area; LD, large deletion; CT, carboxyl-terminal; GH, growth hormone; IGF-1, insulin like growth factor-1; ARSD, Australian Rett Syndrome Database; BMI, body mass index; HT, height.

☆ **Sources of support**: This study was supported by the National Institutes of Health grant 5R01HD043100-05 and NHMRC #303189. Helen Leonard was previously funded by an NHMRC programme grant #353514. Her current funding is from an NHMRC Senior Research Fellowship #572568.

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http://dx.doi.org/10.1016/j.bone.2015.01.023
8756-3282/

Cross-sectional densitometry studies in RTT have shown low bone mineral content (BMC) and areal bone mineral density (aBMD) in children as young as three or four years old [7–9,11]. Even greater reductions in BMC and aBMD z-scores have been observed in older age groups [7,8]. To date, only one study [12], using quantitative ultrasound (QUS), has investigated the changes in bone status in RTT longitudinally. That study assessed bone status in the diaphysis of the proximal phalanx of digits two to five over a three year period in 109 females with RTT aged between 3 and 25 years and 101 age and gender matched controls [12]. Those who were non-ambulant at baseline showed decreasing QUS measurements over time compared to controls, although no change was demonstrated in ambulant subjects [12].

It is well documented that individuals with RTT often have restricted mobility. This was observed in a study of 84 females with RTT, showing that just under half (48%) were immobile or required considerable assistance and almost three quarters (71.5%) either could not stand or needed support to stand [17]. These findings were echoed in our video analysis of the gross motor profile in 99 Australian females with RTT whereby just over half (54.2%) either required assistance to stand or could not stand [18]. Broad relationships between genotype and mobility were also found, with the less severe phenotypic mutations (p.R133C, p.R294X) showing better mobility levels than those with the more severe p.R270X mutation or a large deletion [18]. Subsequently, we found reductions in mobility to be associated with a decrease in lean tissue mass potentially affecting bone mass acquisition [7,19]. However, we also found that low total body (TB) BMC z-scores in RTT normalised when they were adjusted for lean tissue mass [7], suggesting that low muscle volume lean tissue mass may be the mechanism by which low mobility levels precipitated poor bone mineral acquisition.

We know that accretion of bone during childhood is also influenced by hormonal factors including pubertal increases in oestrogen and growth hormone secretion, leading to increased bone mass and cortical thickness [19] and increased bone strength in healthy children [20]. In females both axial skeletal growth and appendicular skeletal growth rapidly increase during early and mid-puberty, followed by a slower period of growth in late puberty [21]. At the onset of puberty, higher levels of oestrogen cause an increase in the secretion of growth hormone (GH) by the anterior pituitary gland, which leads in turn to the generation of insulin like growth factor I (IGF-1). Oestrogen decreases bone remodelling (resorption) by reducing osteoclast numbers, whilst having an anabolic effect on osteoblasts leading to net bone modelling at the endosteum and inhibition at the periosteum [22]. The actions of GH and IGF-1 lead to linear growth of bone by stimulation at the growth plate and also to increased periosteal apposition. During the latter stages of puberty, high oestrogen levels close the growth plate by causing apoptosis of chondrocytes, thereby halting linear growth. At this stage, oestrogen also increases the activity of osteoblasts, having a positive influence on trabecular bone formation [22].

The normal progression of puberty is an essential component of bone development in childhood and adolescence, with an estimated 40% of total bone mass accumulating during the pubertal years, leading to increased BMD and BMC values [23]. Between the ages of 6 and 16 years, total body BMC increases by a factor of two and a half and aBMD doubles mostly from increases in bone size [21]. In RTT, our recent study showed that the median age of onset of menarche was slightly delayed compared to available normative data [24].

The acquisition of bone mass and density is clinically important and in RTT, may have a bearing on increased risk of fracture [25]. This study investigated longitudinal changes in bone mass and density in individuals with RTT over a three to four year period and the influence of lean tissue mass and menstrual status on these parameters.

Methods

Study population

Female participants were sourced from the population based Australian Rett Syndrome Database (ARSD), established in 1993 [25]. For both baseline [7] and follow-up densitometry measurements, appointments were organised at the most convenient location following informed consent procedures. The follow-up scans were performed at the same location as the baseline bone measurements. Ethics approvals were provided by Princess Margaret Hospital in Western Australia, the Children's Hospital at Westmead in New South Wales, Monash Medical Centre in Victoria, Royal Brisbane and Women's Hospital in Queensland and Benson Radiology in South Australia.

Densitometry scans

Densitometry was performed and analysed as in our initial cross-sectional study [7]. Outcome measures were areal bone mass density (aBMD) (g/cm^2) at the lumbar spine (LS) (L2–L4) and total body (TB), and bone mineral composition (BMC) (grammes) at the TB. The TB BMC was further analysed after adjustments for height and lean tissue mass (LTM) (grammes) were made [26]. Height adjusted LTM and bone area (BA) were also assessed. Bone mineral apparent density (BMAD) (g/cm^3) was calculated, as a measure of volumetric density at the LS [26]. All scans affected by movement were excluded from the analysis, as were lumbar bone measures in the presence of spinal rods. Densitometry analyses at baseline and follow-up were performed by one trained operator as previously described [7], and z-scores calculated using Australian normative data [27].

Australian Rett Syndrome Database Questionnaire Data

Information on each individual with RTT was sourced from questionnaires completed by their caregivers and/or clinicians, upon registration into the ARSD and at two to three year intervals thereafter [25]. Mutation type, diagnosis of epilepsy, mobility level, fracture history and menstrual stage were extracted from questionnaires completed nearest to the date of baseline and follow-up scans. Mobility level was classified as “walks unaided” (level 1), “walks with a degree of unsteadiness or with assistance” (level 2) or “does not walk/wheelchair dependent” (level 3). Cases were categorised into one of three menstrual groups; those who had achieved menses more than six months prior to their baseline scan (post-menarcheal), within six months of their baseline scan or between their first and second scans (menarcheal) and those who had not achieved menses during the study period (pre-menarcheal). *MECP2* mutation type was categorised into five groups. The “Mild”, “Mixed” and “Severe” groups were based on general severity of phenotype [2]. The mild mutation group included late carboxyl-terminal truncations (CT), p.R133C, p.R294X and the p.R306C mutation types. The mixed mutation group consisted of p.R106W, p.R168X and p.T158M mutations. Large deletions (LD), p.R255X and p.R270X were placed in the most severe mutation group. The fourth category, referred to as the “Other” group, included the less common *MECP2* gene mutations (p.R306H, p.S134C, p.P152R, p.P255R, early truncating and exon one). The fifth group contained those without a *MECP2* mutation.

Age in years was calculated at the time midway between the baseline and follow-up scans, and then classified into four age groups (4–<10, 10–<15, 15–20 and >20 years). The frequency of fracture was categorised as no fracture history, one fracture, or more than one fracture from birth until the time of the follow-up scan.