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Bone growth in juvenile rhesus monkeys is influenced by 5HTTLPR polymorphisms and interactions between 5HTTLPR polymorphisms and fluoxetine

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Abstract

Male rhesus monkeys received a therapeutic oral dose of the selective serotonin reuptake inhibitor (SSRI) fluoxetine daily from 1 to 3 years of age. Puberty is typically initiated between 2 and 3 years of age in male rhesus and reproductive maturity is reached at 4 years. The study group was genotyped for polymorphisms in the monoamine oxidase A (MAOA) and serotonin transporter (SERT) genes that affect serotonin neurotransmission. Growth was assessed with morphometrics at 4 month intervals and radiographs of long bones were taken at 12 month intervals to evaluate skeletal growth and maturation. No effects of fluoxetine, or MAOA or SERT genotype were found for growth during the first year of the study. Linear growth began to slow during the second year of the study and serotonin reuptake transporter (SERT) long polymorphic region (5HTTLPR) polymorphism effects with drug interactions emerged. Monkeys with two SERT 5HTTLPR L alleles (LL, putative greater transcription) had 25–39% less long bone growth, depending on the bone, than monkeys with one S and one L allele (SL). More advanced skeletal maturity was also seen in the LL group, suggesting earlier onset of puberty. An interaction between 5HTTLPR polymorphisms and fluoxetine was identified for femur and tibia growth; the 5HTTLPR effect was seen in controls (40% less growth for LL) but not in the fluoxetine treated group (10% less growth for LL). A role for serotonin in peripubertal skeletal growth and maturation has not previously been investigated but may be relevant to treatment of children with SSRIs.

Keywords

fluoxetine; serotonin; rhesus; femur; skeletal maturation; height

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1. Introduction

Fluoxetine is a selective serotonin reuptake inhibitor (SSRI) approved by FDA and used in children for depression and obsessive compulsive disorder [1–4]. It is also used as therapy for behavioral symptoms in autism, attention deficit hyperactivity disorder (ADHD), conduct disorders, and anxiety disorders [3–6]. In nonhuman primates, fluoxetine has proven effective in treating stereotypy and self-injurious behavior [7–9], and has been used in experimental studies with translation to humans [10–18].

Effects of childhood fluoxetine treatment on linear growth have been suggested. An effect of fluoxetine on linear (height) growth was reported in a case series [19] as well as in a 19-week clinical trial of fluoxetine treatment in children [1]. SSRIs may play a role in linear growth through serotonin regulation of the production and release of growth hormone (GH) in the hypothalamic-pituitary system [20], but also through direct local effects of serotonin on bone as reflected in bone density [16–18]. Serotonin transporters and serotonin receptors are known to be expressed in bone and a role for serotonin in bone metabolism and bone density has been demonstrated in cell culture [21] and in rodent models using SSRI treatment and serotonin transporter gene (*Sert*, *Slc6a4*) knock out mice [22, 23]. Also, serotonin transporter (SERT) serotonin reuptake transporter long polymorphic region (5HTTLPR) polymorphisms in humans have been shown to interact with SSRIs in influencing bone mineral density in children [24] and adults [25].

In this study, doses in the low therapeutic dose range were used to assess short and long term effects of chronic fluoxetine treatment in juvenile rhesus monkeys, including effects on cognitive performance, social interaction, activity regulation, and linear growth. Juvenile monkeys have become an important model for investigating the effects of childhood psychotropic drug administration including studies of methylphenidate [26–29], respirodone and quetiapine [30, 31] and fluoxetine [12]. Our study included noninvasive morphometric measures and radiographs of the long bones that provide information relevant to effects of fluoxetine and genes relevant to serotonin systems on linear growth during childhood. Juvenile rhesus monkeys were assigned to fluoxetine and vehicle groups to balance two naturally occurring gene polymorphisms that affect serotonin system function, monoamine oxidase A (MAOA) and 5HTTLPR. The influence on growth of fluoxetine, serotonin related polymorphisms and their interactions can be studied with this design.

2. Materials and Methods

2.1 Assurance of compliance with animal codes

All procedures followed the Guide for the Care and Use of Laboratory Animals of the National Research Council. The California National Primate Research Center (CNPRC) is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. Protocols for this project were approved prior to implementation by the UC Davis Institutional Animal Care and Use Committee.

2.2 Design

Thirty two male rhesus macaques born during March, April and May 2011 were selected from the outdoor colony at CNPRC. Infants were excluded from consideration for low weight at birth or three months of age, history of diarrhea, or BioBehavioral Assessment (BBA) scores greater or less than 2 standard deviations from colony mean. (The BBA is a brief (one-day) assessment of temperament and stress responsiveness routinely conducted on 3–4 month old infants at CNPRC.) Infants were transferred to indoor caging at 9–11 months of age (post-weaning age) in two age-based cohorts. They were assigned to treatment groups (fluoxetine, vehicle, n=16/group) randomly balancing for size, cage of origin, MAOA polymorphism genotype and 5HTTLPR polymorphism genotype.

2.3 Animal husbandry

Subjects were pair housed with a compatible age mate in a double cage in a cageroom with two tiers restricted to the study protocol. A divider could be inserted to separate the cagemates for observation, treatment and behavioral testing. Standard husbandry and health care according to CNPRC protocols included: 12/12 (0600–1800) light cycle, cage cleaning daily and change to freshly sterilized cages biweekly, automatic watering with demineralized water, twice daily feeding with Lab Diet #5047 (St. Louis, MO), daily foraging enrichment and twice weekly fresh produce distribution, enrichment with toys inside the cage and mirrors outside the cage, a regular schedule of observation for stereotypy and self-injurious behavior, and daily health check with veterinarian follow-up. Monkeys of concern for weight gain were supplemented with biscuits soaked in commercial nutritional drink and protein bars. Monkeys with persistent diarrhea were treated with oral Tylosin (20 mg/kg). Ketamine (10 mg/kg i.m.) and dexmedetomidine (.0075–.0015 mg/kg i.m., with atipamezole reversal) were given for sedation during morphometric exams and radiography.

2.4 Genotyping

Genotyping for variable number of tandem repeat (VNTR) polymorphisms of MAOA and SERT 5HTTLPR polymorphisms is conducted on most infants in the CNPRC colony at 3–4 months of age with results posted to the colony database. Genotyping is conducted by the Veterinary Genetics Laboratory at UC Davis using polymerase chain reaction (PCR) with primers MAOA-Forward: CAGAAACATGAGCACAAACG (FAM labeled), MAOA-Reverse: TACGAGGTGTCGTCCAAGTT, SERT-Forward GGC GTT GCC GCTCT GAATGC and SERT reverse GAGGGACTGAGCTGGACAACCAC [32, 33]. The MAOA polymorphisms were characterized for high and low transcription rates in rhesus [34], and 5HTTLPR polymorphisms as originally classified for high (long, L) or low (short, S) transcription in humans by Lesch [35] and extended to similar polymorphisms in rhesus [36]. MAOA is X-linked, resulting in two male genotypes, “high” and “low”. 5HTTLPR genotypes based on the allele polymorphisms were “LL”, “LS” and “SS”. The fluoxetine and vehicle groups contained 8 monkeys each with low and high MAOA polymorphism genotypes. The three 5HTTLPR genotypes were distributed as follows: fluoxetine group, LL=8, SL=6, SS=2; vehicle group, LL=9, SL=5, SS=2.

2.5 Fluoxetine dosing

Fluoxetine hydrochloride, obtained as a pediatric solution (20 mg/5 ml, Patterson Veterinary Supply, Inc., Devens, MA), was diluted 1:1 with commercial flavored syrup or baby food prior to administration. After training, fluoxetine was administered via a dosing syringe which was presented at the home cage at the same time each day. Monkeys came forward for dosing, the syringe was emptied into their mouth followed by a “chaser” to ensure swallowing. A therapeutic fluoxetine dose of 2 mg/kg/day was based on literature from children and macaque monkeys and on an acute pharmacokinetic/pharmacodynamic study to confirm internal dose [37]. Dosing was initiated at one year of age at 1.6 mg/kg/day while animals were adjusting to indoor housing and syringe dosing and adjusted to 2.4 mg/kg/day after 11 months. The final dose was confirmed to be within the therapeutic range under the chronic dosing conditions. Dosing continued until three years of age.

2.6 Morphometric measures

Morphometric measures, including body weight, were taken under ketamine anesthesia (10 mg/kg, i.m.) at four month intervals. Crown-rump length (sitting height) was measured using a custom designed measuring board with embedded ruler from the crown of the head to the rump, with the animal in a natural curved position. Standing height is not an appropriate measure in quadruped monkeys. A flexible tape measure was used to assess head circumference (placed horizontally around the brow ridge immediately above the ears) and arm circumference (wrapped around the midpoint of the bicep). Head length and width were measured with Vernier calipers. For length, the tips of the calipers were placed on the brow line just above and between the eyes and on opisthocranium (posterior most point of the cranium). Head width was measured across the head from ear to ear, immediately above the anterior portion of the ears. Skinfold thicknesses were taken with Harpenden calipers at four sites (tricep, mid-scapular region, supriliac crest, mid-thigh) and averaged, and foot length was measured from the end of the longest toe to the back of the heel. All limb measures were taken on the left side. The exams were scheduled from the onset of dosing and all animals were within ± 2 weeks of age at the time of the exams. Morphometric measurements were made in triplicate for seven animals for reliability purposes. The measurements were taken on the same day, after repositioning. Percent error was calculated as the difference between the maximum and the minimum, divided by the mean and multiplied by 100. Average percent error for these measures was 3.41%, with a range from 0–16.22% (highest error reported for skin folds).

2.7 Long bone measures

Digital radiographs of long bones (femur, tibia, radius, ulna) were taken immediately after morphometric measurements at three timepoints: prior to initiation of dosing and after one and two years of dosing. Animals were further sedated for radiographs with ketamine (10 mg/kg, i.m.) and dexmedetomidine (0.0075–0.015 mg/kg, i.m.) followed by atipamezole reversal at the completion of procedures. Radiograph machine settings were determined by the veterinary staff for adequate orthopedic evaluation (Imaging Dynamics Company, Ltd. XPLOER, Calgary, Alberta, Canada for baseline timepoint, InnoVet Select System, Chicago, IL for one and two year timepoints). Two radiographs were taken of each animal,

one for the forelimbs and one for the lower limbs. For x-rays of the forelimbs, animals were placed prone with limb outstretched and elbows slightly bent such that the full length of the limb, from humeral head to finger tips, was visible in one view. For radiograph of the lower limb, animals were positioned supine with limb extended and feet secured with sand bags and tape. The head of the femur to the tips of the toes were visible in one view.

Left side long bone lengths were measured with rulers included in the software program (eRAD, Greenville, SC, for baseline measures, eFilm Workstation®, Chicago, IL, for one and two year timepoints). The diaphysis of each bone, excluding the epiphyses, was measured by placing a line at the most proximal point of the diaphysis and a second parallel line at the most distal point, and then drawing a perpendicular line between these two points. The measure distance tool contained in the software calculated the length of the bone from this perpendicular line (see Supplementary Figure 1).

2.8 Measures of skeletal maturity and bone accrual

The left radius and femur were measured for bone diameter, cortical thickness and skeletal maturity. Bone diameter and medial and lateral cortical thicknesses were measured using the software measurement tool at the calculated midpoint of the diaphysis. A measure of bone accrual, the cortical index, was calculated as the ratio of medial + lateral cortical thickness to bone diameter [38]. A measure of skeletal maturity was calculated as the ratio of the distal epiphyseal width and the distal metaphyseal width [39]. Both the distal epiphyseal and metaphyseal widths were measured at the widest part with lines parallel to each other (see Supplementary Figure 1). Bone length, accrual and maturity measurements were duplicated for seven animals for reliability purposes. These measurements were done on the same radiograph for each animal, one week apart. Percent error was calculated as the difference between the two measures divided by the mean, multiplied by 100. The range of error was 0.1–1.4%, with an average error of 0.7%. The lowest percent error was seen in the long bone lengths.

2.9 Alkaline phosphatase

The Clinical Laboratories at CNPRC analyzed serum samples from the two year dosing timepoint (three years of age) for total alkaline phosphatase via the automated AU480 Chemistry Analyzer (Beckman Coulter, Inc., Brea, CA). This assay was added to the study because fluoxetine was found to cause a decrease in alkaline phosphate in a 19-week clinical trial in children [1].

2.10 Statistical analysis

Growth and morphometric measures were screened prior to analysis for influence of background variables (age cohort, cage location, health history) which were included as covariates if $p < 0.12$. Differences in growth rates between the two years of the study were first examined. Effects of fluoxetine, SERT 5HTTLPR polymorphisms, and MAOA polymorphisms on growth during each year of the study were then determined by multivariate regressions. These analyses detected no consistent effects of the MAOA genotype and this variable was eliminated in the final analyses. Two-way ANOVAs (fluoxetine, SERT) were then conducted on all endpoints. The SERT variable was analyzed

as two levels (LL, SL) with SS animals omitted because there were only 4 animals with SS genotype. Significant interaction terms were followed with planned comparisons of SERT subgroups within fluoxetine/vehicle groups. In addition to two-way ANOVAs, long bone length measures were also analyzed via Repeated Measures ANOVA (RMANOVA) across all four bones (radius, ulna, femur, tibia) and for arm (radius, ulna) and leg (femur, tibia) bones separately. The threshold for identifying statistical significance was $p < 0.05$ with two-tailed testing.

3. Results

All subjects were in good health throughout the study. A daily health check performed by the animal care staff did not report any symptoms requiring veterinary intervention. During the study four animals were given additional nutritional supplements (commercial liquid nutritional supplements and commercial protein bars) to promote weight gain and four animals were given Tylosin to treat diarrhea in accord with CNPRC SOPs. Serum alkaline phosphatase measured at 3 years of age as a potential reflection of bone metabolism [1] showed no effect of fluoxetine treatment ($p = 0.728$) or SERT genotype ($p = 0.241$).

Data were analyzed separately for the two years of the study because of the difference in fluoxetine doses, differences in growth rate and also because of the onset of puberty in the second year. Puberty in male rhesus, as indexed by onset of pulsatile LH release from the pituitary, begins between two and three years of age (onset average 29.5 months stabilizing at 32 months of age [40] or 118 ± 9 weeks [41]). Yearly growth was calculated as the difference between values at one and two years of age and the difference between values at two and three years of age. Figure 1 shows patterns of growth during the two years of the study in the fluoxetine and vehicle control groups for selected measures. Fluoxetine did not have a significant effect on ponderal growth (weight, arm circumference, head circumference), nor linear growth (sitting height).

Growth in the first year of the study was not affected by fluoxetine with the exception of a trend toward greater increase in head measurements in the fluoxetine treated group, which was significant for the head circumference measure ($p = 0.041$) (Table 1). Head growth was significantly *lower* in the fluoxetine group in the second year as reflected in head circumference ($p = 0.021$) and head width ($p = 0.031$).

A slowing in growth rate primarily restricted to linear growth and, in particular, long bone growth (Table 2), was seen in the group as a whole during the second year of the study (2–3 years of age). The reduction in long bone growth was proportionally greater for leg bones (femur 40%, tibia 50%) than for the arm bones (radius 30%, ulna 30%). Trunk length (crown-rump length) growth was 27% slower in the second year. In contrast, weight gain was greater the second year than the first. Skinfold thickness, a measure of subcutaneous fat, increased during the first year but did not change significantly the second year. There were no significant differences in growth by year for foot length or arm circumference, both of which increased significantly during both years of the study (Table 3).

Linear growth was influenced by SERT 5HTTLPR genotype during the second year of the study (Table 2). The LL 5HTTLPR genotype animals showed (25–39%) less linear growth than the SL genotype as seen in long bones. Crown-rump length (sitting height) showed a similar pattern as the long bones, but the 5HTTLPR effect was not significant (Table 3; $p=0.077$). 5HTTLPR effects on growth were not seen for other morphometric measures. Although trends for greater increase in weight ($p=0.076$) and head circumference ($p=0.058$) in the SL group were seen, they did not reach significance.

Additionally, an interaction of 5HTTLPR genotype with fluoxetine was seen for the long bone growth in the leg bones only ($p=0.027$). While the control group showed the effect of 5HTTLPR genotype on leg bone growth ($p<0.001$) it was not significant in the fluoxetine group ($p=0.417$). The data for radius and femur are shown in Figure 2.

Absolute bone length at the end of the study also reflected the 5HTTLPR effect on bone growth. When the monkeys were two years of age, the LL group had shorter lengths across all bones ($p=0.049$). The 5HTTLPR effect on absolute bone length was significant for individual arm bones (radius $p=0.022$, Figure 3A; ulna $p=0.031$, data not shown) but not leg bones ($p=0.087$ femur, $p=.077$ tibia). However an interaction with fluoxetine was seen for the femur length at the end of the study (Figure 3A) with a genotype effect in the control group but not the fluoxetine group ($p=0.047$).

To further examine the 5HTTLPR effect on bone, skeletal maturity and cortical thickness were measured in radiographs of the radius and the femur. Skeletal maturity (ratio of epiphyseal to metaphyseal widths) increased significantly in both radius ($p<0.001$) and femur ($p<0.001$) across the study but was generally lower in the radius. This advanced measure of maturity in the femur is consistent with an earlier slowing of growth in the femur compared to the radius as seen previously in studies of age at epiphyseal closure in rhesus [42, 43]. Skeletal maturity measures of the femur showed a 5HTTLPR genotype effect and were more advanced in the LL group at 3 years of age ($F=4.72$, $p=0.040$). Skeletal maturity measures of the radius were not advanced in the LL group at 3 years of age (Fig 3B).

Bone accrual in the femur, as indicated by a high ratio of the cortical bone thickness to the diameter of the bone, was not significantly affected by LL genotype at 3 years of age, but analysis across ages showed that it was consistently and marginally significantly greater in the LL than the SL group from the time of baseline measure ($F=4.31$, $p=0.048$) and did not change across ages ($p=0.219$) (Figure 4A). In contrast, bone accrual of the radius did increase significantly across ages ($F=40.86$, $p<0.001$). Bone accrual of the radius was not affected by genotype across all ages, but was greater in the LL group at 3 years of age ($F=5.30$, $p=0.030$). Interactions with fluoxetine were not seen for bone accrual measures (Figure 4B).

4. Discussion

The current study provides a controlled experiment in an appropriate animal model to study fluoxetine effects and the impact of polymorphisms on juvenile growth. The major finding was an influence of 5HTTLPR genotype on skeletal growth and maturation in the early

pubertal period. Animals with the LL genotype slowed their linear growth and had more advanced skeletal maturity suggesting faster pubertal progression. Faster pubertal maturation associated with LL genotype was also suggested in a study of female monkeys [44]. The endpoint in that study was age at first ovulation, and earlier puberty was accompanied by higher nocturnal serum GH. No relevant information on 5HTTLPR genotype and pubertal growth in humans was located.

In general, fluoxetine was not found to impair juvenile growth. Information on fluoxetine effects on growth in children is suggestive but very limited. A case series described four pubertal children being treated with fluoxetine or fluvoxamine for obsessive-compulsive disorder (OCD) or Tourette syndrome and referred for growth deficits. SSRI effects on height growth velocity were inferred from changes detected at initiation/discontinuation of treatment in 3 cases [19]. Nilsson et al., [1] reporting a randomized, controlled clinical trial of fluoxetine for depression in children, described less height growth and weight gain after 19 weeks of treatment compared to controls. The authors expressed “limited” confidence in this finding and no rebound growth was noted when fluoxetine treatment was discontinued in a subgroup of the previously dosed children [45]. The finding of reduced growth was also described in the original study report submitted to FDA in connection with approval of Prozac® for use in children [46]. Notably, the 2003 approval letter from FDA described the commitment of the applicant to conduct a prospective post-marketing study of growth, but we were not able to locate such a study.

Studies in children are complicated by age range of the population under study, confounding by indication, polypharmacy, dose variation and a variety of uncontrolled environmental and genetic factors that can be controlled in a nonhuman primate study. Nonhuman primates can model the prolonged juvenile period, the initiation and progress of puberty and importantly, the epiphyseal closure that terminates long bone growth in primates [41–43]. The present study is the first to provide evaluation of growth during chronic fluoxetine treatment of juvenile nonhuman primates. Shrestha et al. [12] treated juvenile rhesus monkeys with 3 mg/kg/d fluoxetine from 2 to 3 years of age but did not report any data or findings concerning growth. Studies of fluoxetine treatment of juvenile and adolescent rodents seldom report body weight data and do not report linear growth measures. One study with intraperitoneal (i.p.) administration of a behaviorally active dose of fluoxetine during juvenile development of rats did document substantially lower body weight gain [47].

In the present study, a wide range of growth measures were obtained over a two year period of fluoxetine treatment. The only growth measures showing a main effect of fluoxetine across all genotypes were head measures. A lag in growth in the peripubertal period was suggested with faster growth than controls in the first year and slower growth the second year, resulting in equivalent growth across the study. The effect of fluoxetine on head growth is difficult to interpret due to lack of context in the literature. One paper has reported a specific effect of prenatal fluoxetine on fetal head growth [48] but this effect did not emerge from meta-analysis across prenatal fluoxetine studies [48, 49]. The different direction of the effect of fluoxetine, increasing growth during the first year and more strongly decreasing growth during second year, requires replication. Concurrent measures of brain growth during that period would be valuable.

5HTTLPR polymorphisms have been studied in connection with brain growth in children with the autism symptom of enhanced early brain growth. A study in 2- and 4-year-olds suggested more rapid growth in SL genotype autistic children [50], but this was not seen in adults/adolescents in another study [51]. Larger brains were part of the phenotype of transgenic mice heterozygous for SERT knock-out (KO) [52], consistent with less SERT expression in the SL 5HTTLPR genotype. MAOA [53] and 5HT [54] genes have also been implicated in the general hypothesis that excess serotonin, due to increased production, decreased metabolism, or decreased reuptake, accelerates brain growth during development. Greater head growth in the SL group of the present study is consistent with this potential effect of excess serotonin.

Although a main effect of fluoxetine was not seen on linear or ponderal growth, an interaction of fluoxetine with 5HTTLPR polymorphism of the SERT gene was seen for linear growth during the early pubertal period, specifically leg bone growth. Growth of each of the 4 bones measured was slower in the second year of the study in animals with LL 5HTTLPR polymorphism genotypes than those with SL genotypes. However, for the femur and tibia, the genotype effect did not occur within the fluoxetine group. Post hoc tests of fluoxetine effects within genotype groups were not significant. The pattern of means suggests the hypothesis that SL monkeys failed to maintain growth rate when treated with fluoxetine. Calarge [24] reported an interaction of 5HTTLPR genotype and SSRIs on bone mineral density in that lower bone density was seen specifically in the SL genotype in that study but a similar interaction for bone length was not investigated. Although the interaction between fluoxetine and 5HTTLPR genotype in the present study was only significant for bone length measures, similar interaction trends were suggested for other linear growth measures.

The data did not suggest an effect of fluoxetine on bone accrual as indexed by bone cortical thickness measured from radiographs. Information on fluoxetine effects on bone mineralization in children is also limited but suggestive. Calarge [24] studied a group of respirodone treated boys (average age 12 years) half of whom were also treated with SSRIs (agents not specified). In regression analysis, SSRI treatment was associated with lower trabecular volumetric bone mineral density (vBMD) in the distal radius and a trend for effects on total vBMD (trabecular and cortical). BMD of the lumbar spine was not affected but SSRI treatment showed some association with an age-corrected lumbar BMD z-score based on historical control data. In addition, in a reanalysis of the data which included 5HTTLPR polymorphism genotyping, an interaction between 5HTTLPR polymorphisms and SSRI treatment was found for the total lumbar BMD z-score [24]. Effects of SSRIs on chondrocytes and cartilaginous tissue growth have not been studied, but serotonin receptors have been identified in embryonic and adult cartilaginous tissue [55, 56].

In general, epiphyseal closure and bone mineralization in the peripubertal period are not known to be temporally synchronous or regulated by the same mechanisms [57, 58]. However, blockade of serotonin reuptake at the cellular membrane could affect several regulatory processes including serotonin action on osteoblasts, as well as effects on the onset of puberty via serotonin regulation of pituitary hormone production and release [41, 57–61]. LL deficits in linear growth seen in the current study are consistent with the idea that

serotonin produced in the gut suppresses bone formation [60] but could also be centrally mediated [62].

Chronic fluoxetine treatment has been shown to produce a long-term upregulation of the serotonin transporter in the brain after discontinuation of treatment [12]. The interaction between 5HTTLPR and fluoxetine in control of linear growth of the tibia and femur in juvenile monkeys, and bone mineralization of the tibia in children, suggests the possibility of a common site of action.

The investigation of hypotheses in controlled experiments in animal models is critical to establishing cause and effect relationships and identifying mechanisms but, inevitably, results in limitations in generalization. In the present study this applies to generalization to girls, to other fluoxetine doses, to clinical populations with varying diagnoses, and to children receiving multiple therapies for behavioral and other disorders with differing ages of onset of treatment, etc. At the same time, the integration of human, rodent and nonhuman primate studies suggests that the research topic of SSRI treatment and linear growth requires further investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Nilsson M, Joliat MJ, Miner CM, Brown EB, Heiligenstein JH. Safety of subchronic treatment with fluoxetine for major depressive disorder in children and adolescents. *J Child Adolesc Psychopharmacol*. 2004; 14(3):412–417. [PubMed: 15650497]
2. Geller DA, Hoog SL, Heiligenstein JH, Ricardi RK, Tamura R, et al. Fluoxetine treatment for obsessive-compulsive disorder in children and adolescents: a placebo-controlled clinical trial. *J Am Acad Child Adolesc Psychiatry*. 2001; 40(7):773–779. [PubMed: 11437015]
3. Riggs PD, Mikulich-Gilbertson SK, Davies RD, Lohman M, Klein C, et al. A randomized controlled trial of fluoxetine and cognitive behavioral therapy in adolescents with major depression, behavior problems and substance use disorders. *Arch Pediatr Adolesc Med*. 2007; 161(11):1026–1034. [PubMed: 17984403]
4. Quintana H, Butterbaugh GJ, Purnell W, Layman AK. Fluoxetine monotherapy in attention-deficit/hyperactivity disorder and comorbid non-bipolar mood disorders in children and adolescents. *Child Psychiatry Hum Dev*. 2007; 37(3):241–253. [PubMed: 17103304]
5. Birmaher B, Axelson DA, Monk K, Kalas C, Clark DB, et al. Fluoxetine for the treatment of childhood anxiety disorders. *J Am Acad Child Adolesc Psychiatry*. 2003; 42(4):415–423. [PubMed: 12649628]
6. Hollander E, Phillips A, Chaplin W, Zagursky K, Novotny S, et al. A placebo controlled crossover trial of liquid fluoxetine on repetitive behaviors in childhood and adolescent autism. *Neuropsychopharmacology*. 2005; 30(3):582–589. [PubMed: 15602505]

7. Fontenot MB, Musso MW, McFatter RM, Anderson GM. Dose-finding study of fluoxetine and venlafaxine for the treatment of self-injurious and stereotypic behavior in rhesus macaques (*Macaca mulatta*). *J Am Assoc Lab Anim Sci*. 2009; 48(2):176–184. [PubMed: 19383215]
8. Fontenot MB, Padgett EE 3rd, Dupuy AM, Lynch CR, De Petrillo PB, et al. The effects of fluoxetine and buspirone on self-injurious and stereotypic behavior in adult male rhesus macaques. *Comp Med*. 2005; 55(1):67–74. [PubMed: 15766212]
9. Sawyer EK, Howell LL. Pharmacokinetics of fluoxetine in rhesus macaques following multiple routes of administration. *Pharmacology*. 2011; 88(1–2):44–49. [PubMed: 21757974]
10. Bogdan R, Fitzgibbon H, Woolverton WL, Bethea CL, Iyo AH, et al. 5-HTTLPR genotype and gender, but not chronic fluoxetine administration are associated with cortical TREK1 protein expression in rhesus macaques. *Neurosci Lett*. 2011; 503(2):83–86. [PubMed: 21871532]
11. Shiue CY, Shiue GG, Cornish KG, O'Rourke MF. PET study of the distribution of [¹¹C]fluoxetine in a monkey brain. *Nucl Med Biol*. 1995; 22(5):613–616. [PubMed: 7581171]
12. Shrestha SS, Nelson EE, Liow JS, Gladding R, Lyoo CH, et al. Fluoxetine administered to juvenile monkeys: effects on the serotonin transporter and behavior. *Am J Psychiatry*. 2014; 171(3):323–331. [PubMed: 24480874]
13. Anderson GM. Peripheral and central neurochemical effects of the selective serotonin reuptake inhibitors (SSRIs) in humans and nonhuman primates: assessing bioeffect and mechanisms of action. *Int J Dev Neurosci*. 2004; 22(5–6):397–404. [PubMed: 15380838]
14. Anderson GM, Barr CS, Lindell S, Durham AC, Shifrovich I, et al. Time course of the effects of the serotonin-selective reuptake inhibitor sertraline on central and peripheral serotonin neurochemistry in the rhesus monkey. *Psychopharmacology*. 2005; 178(2–3):339–346. [PubMed: 15452685]
15. Anderson GM, Bennett AJ, Weld KP, Pushkas JG, Ocame DM, et al. Serotonin in cisternal cerebrospinal fluid of rhesus monkeys: basal levels and effects of sertraline administration. *Psychopharmacology*. 2002; 161(1):95–99. [PubMed: 11967636]
16. Clarke AS, Ebert MH, Schmidt DE, McKinney WT, Kraemer GW. Biogenic amine activity in response to fluoxetine and desipramine in differentially reared rhesus monkeys. *Biol Psychiatry*. 1999; 46(2):221–228. [PubMed: 10418697]
17. Clarke AS, Kraemer GW, Kupfer DJ. Effects of rearing condition on HPA axis response to fluoxetine and desipramine treatment over repeated social separations in young rhesus monkeys. *Psychiatry Res*. 1998; 79(2):91–104. [PubMed: 9705048]
18. Laudenslager ML, Clarke AS. Antidepressant treatment during social challenge prior to 1 year of age affects immune and endocrine responses in adult macaques. *Psychiatry Res*. 2000; 95(1):25–34. [PubMed: 10904120]
19. Weintrob N, Cohen D, Klipper-Aurbach Y, Zadik Z, Dickerman Z. Decreased growth during therapy with selective serotonin reuptake inhibitors. *Arch Pediatr Adolesc Med*. 2002; 156(7):696–701. [PubMed: 12090838]
20. Musumeci G, Trovato FM, Avola R, Imbesi R, P C. Serotonin/growth hormone/insulin like growth factors axis on pre- and post-natal development: a contemporary review. *OA Anatomy*. 2013; 1(1):12.
21. Bliziotis M. Update in Serotonin and Bone. *J Clin Endocrinol Metab*. 2010; 95(9):4124–4132. [PubMed: 20823465]
22. Warden SJ, Hassett SM, Bond JL, Rydberg J, Grogg JD, et al. Psychotropic drugs have contrasting skeletal effects that are independent of their effects on physical activity levels. *Bone*. 2010; 46(4):985–992. [PubMed: 20060080]
23. Warden SJ, Robling AG, Sanders MS, Bliziotis MM, Turner CH. Inhibition of the serotonin (5-hydroxytryptamine) transporter reduces bone accrual during growth. *Endocrinology*. 2005; 146(2):685–693. [PubMed: 15539550]
24. Calarge CA, Ellingrod VL, Zimmerman B, Bliziotis MM, Schlechte JA. Variants of the serotonin transporter gene, selective serotonin reuptake inhibitors and bone mineral density in risperidone-treated boys: a reanalysis of data from a cross-sectional study with emphasis on pharmacogenetics. *J Clin Psychiatry*. 2011; 72(12):1685–1690. [PubMed: 22244026]

25. Garfield LD, Muller DJ, Kennedy JL, Mulsant BH, Reynolds CF 3rd, et al. Genetic variation in the serotonin transporter and HTR1B receptor predicts reduced bone formation during serotonin reuptake inhibitor treatment in older adults. *World J Biol Psychiatry*. 2014; 15(5):404–410. [PubMed: 24074042]
26. Rodriguez JS, Morris SM, Hotchkiss CE, Doerge DR, Allen RR, et al. The effects of chronic methylphenidate administration on operant test battery performance in juvenile rhesus monkeys. *Neurotoxicol Teratol*. 2010; 32(2):142–151. [PubMed: 19737611]
27. Morris SM, Dobrovolsky VN, Shaddock JG, Mittelstaedt RA, Bishop ME, et al. The genetic toxicology of methylphenidate hydrochloride in non-human primates. *Mutat Res*. 2009; 673(1): 59–66. [PubMed: 19135169]
28. Mattison DR, Plant TM, Lin HM, Chen HC, Chen JJ, et al. Pubertal delay in male nonhuman primates (*Macaca mulatta*) treated with methylphenidate. *Proc Natl Acad Sci U S A*. 2011; 108(39):16301–16306. [PubMed: 21930929]
29. Soto PL, Wilcox KM, Zhou Y, Kumar A, Ator NA, et al. Long-term exposure to oral methylphenidate or dl-amphetamine mixture in peri-adolescent rhesus monkeys: effects on physiology, and behavior and dopamine system development. *Neuropsychopharmacology*. 2012; 37(12):2566–2579. [PubMed: 22805599]
30. Mandell DJ, Unis A, Sackett GP. Post-drug consequences of chronic atypical antipsychotic drug administration on the ability to adjust behavior based on feedback in young monkeys. *Psychopharmacology (Berl)*. 2011; 215(2):345–352. [PubMed: 21221533]
31. Sackett G, Unis A, Crouthamel B. Some effects of risperidone and quetiapine on growth parameters and hormone levels in young pigtail macaques. *J Child Adolesc Psychopharmacol*. 2010; 20(6):489–493. [PubMed: 21186967]
32. Capitanio JP, Del Rosso LA, Calonder LA, Blozis SA, Penedo MC. Behavioral effects of prenatal ketamine exposure in rhesus macaques are dependent on MAOA genotype. *Exp Clin Psychopharmacol*. 2012; 20(3):173–180. [PubMed: 22250657]
33. Kinnally EL, Lyons LA, Abel K, Mendoza S, Capitanio JP. Effects of early experience and genotype on serotonin transporter regulation in infant rhesus macaques. *Genes Brain Behav*. 2008; 7(4):481–486. [PubMed: 18081711]
34. Newman TK, Syagailo YV, Barr CS, Wendland JR, Champoux M, et al. Monoamine oxidase A gene promoter variation and rearing experience influences aggressive behavior in rhesus monkeys. *Biol Psychiatry*. 2005; 57(2):167–172. [PubMed: 15652876]
35. Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, et al. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science*. 1996; 274(5292):1527–1531. [PubMed: 8929413]
36. Lesch KP, Meyer J, Glatz K, Flugge G, Hinney A, et al. The 5-HT transporter gene-linked polymorphic region (5-HTTLPR) in evolutionary perspective: alternative biallelic variation in rhesus monkeys. *Rapid communication. J Neural Transm*. 1997; 104(11–12):1259–1266. [PubMed: 9503271]
37. Golub MS, Hogrefe CE. Fluoxetine: juvenile pharmacokinetics in a nonhuman primate model. *Psychopharmacology (Berl)*. 2014
38. Duren DL, Blangero J, Sherwood RJ, Seselj M, Dyer T, et al. Cortical bone health shows significant linkage to chromosomes 2p, 3p, and 17q in 10-year-old children. *Bone*. 2011; 49(6): 1213–1218. [PubMed: 21907839]
39. Roche, A.; Chumlea, WC.; Thissen, D. *Assessing the Skeletal Maturity of the Hand-Wrist: Fels Method*. Springfield: Charles C. Thomas; 1988.
40. Mann DR, Bhat GK, Stah CD, Pohl CR, Plant TM. Induction of a hypothyroid state during juvenile development delays pubertal reactivation of the neuroendocrine system governing luteinising hormone secretion in the male rhesus monkey (*Macaca mulatta*). *J Neuroendocrinol*. 2006; 18(9):662–671. [PubMed: 16879165]
41. Fraser MO, Arslan M, Plant TM. Androgen estrogen treatment alone or in combination differentially influences bone maturation and hypothalamic mechanisms that time puberty in the male rhesus monkey (*Macaca mulatta*). *Pediatr Res*. 2005; 57(1):141–148. [PubMed: 15557106]

42. Silverman S, Morgan JP, Ferron R, McNulty W, Merten D. Radiographic evaluation of appendicular skeletal maturation in the rhesus monkey. *Vet Radiol.* 1983; 24(1):25–34.
43. Van Wagenen G, Asling CW. Roentgenographic estimation of bone age in the Rhesus monkey (*Macaca mulatta*). *Am J Anat.* 1958; 103(2):163–185. [PubMed: 13636987]
44. Wilson ME, Kinkead B. Gene-environment interactions not neonatal growth hormone deficiency time puberty in female rhesus monkeys. *Biol Reprod.* 2008; 78(4):736–743. [PubMed: 18160679]
45. Emslie GJ, Heiligenstein JH, Hoog SL, Wagner KD, Findling RL, et al. Fluoxetine treatment for prevention of relapse of depression in children and adolescents: a double-blind placebo-controlled study. *J Am Acad Child Adolesc Psychiatry.* 2004; 43(11):1397–1405. [PubMed: 15502599]
46. Eli Lilly and Company. Clinical Study summary: Study B1Y–MC–HCIU. 2004
47. Iniguez SD, Warren BL, Bolanos-Guzman CA. Short- and long-term functional consequences of fluoxetine exposure during adolescence in male rats. *Biol Psychiatry.* 2010; 67(11):1057–1066. [PubMed: 20172503]
48. El Marroun H, Jaddoe VW, Hudziak JJ, Roza SJ, Steegers EA, et al. Maternal use of selective serotonin reuptake inhibitors, fetal growth and risk of adverse birth outcomes. *Arch Gen Psychiatry.* 2012; 69(7):706–714. [PubMed: 22393202]
49. Wisner KL, Bogen DL, Sit D, McShea M, Hughes C, et al. Does fetal exposure to SSRIs or maternal depression impact infant growth? *Am J Psychiatry.* 2013; 170(5):485–493. [PubMed: 23511234]
50. Wassink TH, Hazlett HC, Epping EA, Arndt S, Dager SR, et al. Cerebral cortical gray matter overgrowth and functional variation of the serotonin transporter gene in autism. *Arch Gen Psychiatry.* 2007; 64(6):709–717. [PubMed: 17548752]
51. Raznahan A, Pugliese L, Barker GJ, Daly E, Powell J, et al. Serotonin transporter genotype and neuroanatomy in autism spectrum disorders. *Psychiatr Genet.* 2009; 19(3):147–150. [PubMed: 19369912]
52. Page DT, Kuti OJ, Prestia C, Sur M. Haploinsufficiency for Pten and Serotonin transporter cooperatively influences brain size and social behavior. *PNAS.* 2009; 106(6):1989–1994. [PubMed: 19208814]
53. Wassink TH, Hazlett HC, Davis LK, Reiss AL, Piven J. Testing for association of the monoamine oxidase A promoter polymorphism with brain structure volumes in both autism and the fragile × syndrome. *J Neurodev Disord.* 2014; 6
54. Flood ZC, Engel DLJ, Simon CC, Negherbon KR, Murphy LJ, et al. Brain growth trajectories in mouse strains with central and peripheral serotonin differences: Relevance to autism models. *Neuroscience.* 2012; 210:286–295. [PubMed: 22450231]
55. Yoo HJ, Yoon SS, Park SY, Lee EY, Lee EB, et al. Gene expression profile during chondrogenesis in human bone marrow derived mesenchymal stem cells using a cDNA microarray. *J Korean Med Sci.* 2011; 26(7):851–858. [PubMed: 21738335]
56. Lauder JM, Wilkie MB, Wu C, Singh S. Expression of 5-HT(2A) 5-HT(2B) and 5-HT(2C) receptors in the mouse embryo. *Int J Dev Neurosci.* 2000; 18(7):653–662. [PubMed: 10978843]
57. Fournier PE, Rizzoli R, Slosman DO, Theintz G, Bonjour JP. Asynchrony between the rates of standing height gain and bone mass accumulation during puberty. *Osteoporos Int.* 1997; 7(6):525–532. [PubMed: 9604047]
58. Bradney M, Karlsson MK, Duan Y, Stuckey S, Bass S, et al. Heterogeneity in the growth of the axial and appendicular skeleton in boys: implications for the pathogenesis of bone fragility in men. *J Bone Miner Res.* 2000; 15(10):1871–1878. [PubMed: 11028438]
59. Oury F, Yadav VK, Wang Y, Zhou B, Liu XS, et al. CREB mediates brain serotonin regulation of bone mass through its expression in ventromedial hypothalamic neurons. *Genes Dev.* 2010; 24(20):2330–2342. [PubMed: 20952540]
60. Yadav VK, Ducy P. Lrp5 and bone formation : A serotonin-dependent pathway. *Ann N Y Acad Sci.* 1192:103–109.
61. Perry RJ, Farquharson C, Ahmed SF. The role of sex steroids in controlling pubertal growth. *Clin Endocrinol.* 2008; 68:4–15.

62. Yadav VK, Oury F, Suda N, Liu ZW, Gao XB, et al. A serotonin-dependent mechanism explains the leptin regulation of bone mass, appetite and energy expenditure. *Cell*. 2009; 138(5):976–989. [PubMed: 19737523]

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Highlights

- Rhesus monkeys were studied during a 2 year period of prepubertal growth.
- Half the monkeys were treated with a therapeutic dose of fluoxetine.
- Prior to puberty long bone growth slowed and skeletal maturity advanced.
- High expression 5HTTLPR polymorphisms led to greater slowing of growth.
- The genetic effect on bone growth was not seen in fluoxetine treated monkeys.

Figure 1. Ponderal (A, B) and linear (C) growth during two years of administration of a therapeutic dose of fluoxetine to juvenile rhesus monkeys (n=16/group). Mean $\pm$ SEM are shown. No main effects of fluoxetine on growth were indicated in either year.

Figure 2.

Effects of SERT 5HTTLPR polymorphisms on linear growth of the radius and femur. Mean $\pm$ SEM is shown. During the second year of dosing, growth slowed and a genotype effect emerged as greater reduction of growth in the LL compared to SL genotype subjects. For the femur the genotype effect was seen in controls but not in the fluoxetine group. No effect of fluoxetine was seen for the radius. LL= putative greater expression, SL=putative lower expression of the serotonin reuptake transporter. Control LL n=9, Control SL n=5, Fluoxetine LL n=8, Fluoxetine SL n=6. Statistics from ANOVA analysis with fluoxetine, genotype and interaction terms and post hoc contrasts. The absolute values of femur and radius length at different ages are shown in Supplementary Figure 2.

Figure 3.

Long bone length (A) and skeletal maturity (B) at the end of the dosing period. For the radius, a genotype effect (LL<SL) was seen for bone length but not for skeletal maturity. Skeletal maturity was more advanced in the femur and the genotype effect (LL>SL) was seen. The genotype effect on femur length was significant in controls, but not in the fluoxetine group. See Figure 2 caption for analysis methods and group sizes.

Figure 4.

Bone accrual in the femur (A) and radius (B) at three timepoints during the dosing period. RMANOVA across all three timepoints demonstrated genotype effect for the femur, with no significant effect of timepoint. For the radius, there was a significant effect of timepoint, with a significant genotype effect at the last timepoint (three years old). See Figure 2 caption for group sizes and analysis methods.

Table 1

Head measure growth. Values are means $\pm$ SEM and represent the change in size during Year 1 and Year 2 of fluoxetine dosing. P-values are from ANOVA with fluoxetine and serotonin transporter genotype as independent variables and include the interaction. LL = putative greater expression, SL = putative lower expression of serotonin reuptake transporter (SERT); FLX p-value = main effect of fluoxetine on growth; SERT p-value = main effect serotonin reuptake transporter genotype on growth; ns = not significant. Comparative group values for other somatic growth measures are shown in Supplementary Table 1.

	LL Flx (n= 8)	LL Control (n=9)	SL Flx (n=6)	SL Control (n=5)	FLX p-value	SERT p-value
Head length (mm)						
Year 1 growth	6.5 $\pm$ 1.0	5.5 $\pm$ 0.5	5.4 $\pm$ 0.6	4.6 $\pm$ 0.7	ns	ns
Year 2 growth	4.7 $\pm$ 0.8	4.8 $\pm$ 0.5	6.2 $\pm$ 1.1	7.4 $\pm$ 0.9	0.086	0.021 *
Head width (mm)						
Year 1 growth	5.7 $\pm$ 0.7	4.6 $\pm$ 0.7	4.9 $\pm$ 0.5	3.3 $\pm$ 1.0	0.086	ns
Year 2 growth	4.5 $\pm$ 0.3	5.5 $\pm$ 0.7	4.9 $\pm$ 0.8	6.9 $\pm$ 0.8	0.031 *	ns
Head circumference (cm) [#]						
Year 1 growth	2.2 $\pm$ 0.2	1.5 $\pm$ 0.2	1.9 $\pm$ 0.3	1.3 $\pm$ 0.4	0.041 *	ns
Year 2 growth	1.6 $\pm$ 0.1	2.2 $\pm$ 0.3	2.1 $\pm$ 0.3	2.8 $\pm$ 0.2	0.019 *	0.058

* ANOVA main effect, $p < 0.05$

[#] Year 1 vs Year 2 growth rate, $p = 0.054$, $Y2 > Y1$

Table 2

Bone length growth. Values are means $\pm$ SEM and represent the change in length during Year 1 and Year 2 of fluoxetine dosing. P-values are from ANOVA with fluoxetine and serotonin transporter genotype as independent variables and include the interaction. See Table 1 for abbreviations.

	LL Flx (n=8)	LL Control (n=9)	SL Flx (n=6)	SL Control (n=5)	FLX p-value	SERT p-value
Radius (mm) ^{§§}						
Year 1 growth	27.6 $\pm$ 1.3	25.6 $\pm$ 1.2	26.1 $\pm$ 3.0	25.9 $\pm$ 1.3	ns	ns
Year 2 growth	17.4 $\pm$ 0.6	17.1 $\pm$ 1.0	22.0 $\pm$ 2.3	21.6 $\pm$ 1.9	ns	0.004 ^{**}
Ulna (mm) ^{§§}						
Year 1 growth	32.5 $\pm$ 1.5	29.6 $\pm$ 1.7	30.6 $\pm$ 3.4	30.2 $\pm$ 4.0	ns	ns
Year 2 growth	19.5 $\pm$ 1.4	20.5 $\pm$ 0.8	25.2 $\pm$ 2.9	25.6 $\pm$ 2.1	ns	0.005 ^{**}
Femur (mm) ^{§§}						
Year 1 growth	36.4 $\pm$ 3.0	35.2 $\pm$ 1.4	36.5 $\pm$ 3.2	34.2 $\pm$ 4.3	ns	ns
Year 2 growth	20.6 $\pm$ 1.5	17.2 $\pm$ 2.2	22.0 $\pm$ 2.5	27.4 $\pm$ 3.5	ns	0.023 [*]
Tibia (mm) ^{§§}						
Year 1 growth	37.6 $\pm$ 2.0	33.7 $\pm$ 1.4	33.2 $\pm$ 3.4	33.4 $\pm$ 4.6	ns	ns
Year 2 growth	16.7 $\pm$ 0.8	13.0 $\pm$ 2.3	19.5 $\pm$ 1.6	22.6 $\pm$ 2.5	ns	0.003 ^{**}

* ANOVA main effect, $p < 0.05$

** ANOVA main effect, $p < 0.01$

^{§§} Year 1 vs Year 2 growth rate, $p < 0.001$, Y2 < Y1

Table 3

Additional growth measures. Values are means $\pm$ SEM and represent the change in growth during Year 1 and Year 2 of fluoxetine dosing. P-values are from ANOVA with fluoxetine and serotonin transporter as independent variables and include the interaction. See Table 1 for abbreviations.

	LL Flx (n=8)	LL Control (n=9)	SL Flx (n=6)	SL Control (n=5)	FLX p- value	SERT p- value
Crown-rump (mm) ***						
Year 1 growth	88 $\pm$ 8	77 $\pm$ 5	84 $\pm$ 6	81 $\pm$ 10	ns	ns
Year 2 growth	58 $\pm$ 2	57 $\pm$ 5	61 $\pm$ 4	70 $\pm$ 6	ns	0.077 $^{#}$
Weight (kg) **						
Year 1 growth	1.39 $\pm$ 0.15	1.28 $\pm$ 0.14	1.19 $\pm$ 0.18	1.36 $\pm$ 0.19	ns	ns
Year 2 growth	1.32 $\pm$ 0.18	1.56 $\pm$ 0.17	1.84 $\pm$ 0.21	1.79 $\pm$ 0.23	ns	0.076 $^{#}$
Skin folds (mm) *						
Year 1 growth	0.3 $\pm$ 0.1	0.4 $\pm$ 0.1	-0.03 $\pm$ 0.1	0.2 $\pm$ 0.2	ns	0.099 $^{#}$
Year 2 growth	-0.2 $\pm$ 0.2	0.1 $\pm$ 0.1	0.04 $\pm$ 0.2	-0.2 $\pm$ 0.2	ns	ns
Arm circumference (cm)						
Year 1 growth	2.5 $\pm$ 0.3	2.0 $\pm$ 0.3	1.3 $\pm$ 0.3	2.0 $\pm$ 0.3	ns	0.049 *
Year 2 growth	2.3 $\pm$ 0.6	1.8 $\pm$ 0.5	2.7 $\pm$ 0.7	2.0 $\pm$ 0.7	ns	ns
Foot length (mm) *						
Year 1 growth	21 $\pm$ 2	17 $\pm$ 2	20 $\pm$ 2	18 $\pm$ 3	ns	ns
Year 2 growth	16 $\pm$ 2	16 $\pm$ 2	17 $\pm$ 2	18 $\pm$ 2	ns	ns

* p<0.05, ANOVA main effect

$^{#}$ 0.10>p>0.05, ANOVA main effect

* p<0.01, Year 1 vs Year 2 growth rate, Y2<Y1

** p<0.001, Year 1 vs Year 2 growth rate, Y2>Y1

*** p<0.0001, Year 1 vs Year 2 growth rate, Y2<Y1

* 0.10>p>0.05, Year 1 vs Year 2 growth rate, Y2<Y1