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ORIGINAL ARTICLE

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Bone mineral density in complete androgen insensitivity syndrome and the timing of gonadectomy

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Summary

Objective: Low bone mineral density (BMD) has been reported in complete androgen insensitivity syndrome (CAIS), but the impact of timing of gonadectomy is not known. We aimed to assess the relationship between age of gonadectomy and BMD in women with CAIS.

Design: Retrospective analysis of pre- and post-gonadectomy parameters in women with CAIS attending an adult Disorders of Sex Development (DSD) clinic in a tertiary centre.

Patients: One hundred and thirteen women with CAIS.

Measurements: Dual-energy X-ray absorptiometry (DXA) before and after gonadectomy; and pre-gonadectomy hormone profile.

Results: Mean BMD was reduced (95% confidence interval); T-score −1.34 (−1.55 to −1.13; *P*<.001) at the lumbar spine and −0.3 (−0.49 to −0.12; *P*=.001) at the hip. There was no relationship between age of gonadectomy and BMD. Thirty-two subjects had BMD measured before or within 2 years of gonadectomy, and mean BMD was reduced (95% CI) at the lumbar spine; T-score: −1.05 (−1.54 to −0.57; *P*<.001), but was normal at the hip; T-score −0.04 (−0.35 to 0.28; *P*=.8). There was no relationship between BMD and history of hernia, testosterone, oestradiol or follicle stimulating hormone levels. Twelve subjects had DXA both before and after gonadectomy, and after 4.3 (1.7-12.8) years, there was no change in BMD.

Conclusions: We found reduced BMD at the spine and hip in subjects with CAIS. We found no relationship between age of gonadectomy and BMD, and we also found no drop in BMD in subjects followed up after gonadectomy.

KEYWORDS

bone mineral density (BMD), complete androgen insensitivity syndrome (CAIS), disorders of sex development (DSD), gonadectomy

1 | INTRODUCTION

First described in 1953, complete androgen insensitivity syndrome (CAIS) is one of the commonest 46, XY disorders of sex development (DSD).[1] The syndrome is caused by loss of function mutations in the androgen receptor and has an estimated incidence of 1-5 per 100 000 births.[2,3] The presentation of CAIS is usually during adolescence with primary amenorrhoea in the setting of normal breast development and absent or sparse pubic and axillary hair. CAIS may also be discovered during infancy when an inguinal hernia or labial swelling is found to contain testicular tissue.

The risk of gonadal tumour is a recognised aspect of the clinical management of CAIS, and it has been standard practice to advise prophylactic gonadectomy as soon as the diagnosis is confirmed. The risk of malignancy in adulthood is estimated to be 14%; however, the risk in paediatric populations is much lower and ranges from 0.8%

to 2.0%, and malignant tumours have not been reported in subjects under the age of 14.[4-7] Consequently some centres and advocacy groups suggest leaving the gonads in situ throughout adolescence.[6] This approach allows spontaneous pubertal development to occur via aromatization of testosterone, and also enables self-determination of decisions at a later age. University College London Hospital (UCLH) is a national referral centre for DSD, and current practice is to recommend gonadectomy after completion of puberty; however, an increasing number of adult women with CAIS elect to defer gonadectomy indefinitely.

Low bone mineral density (BMD) has been reported in subjects with CAIS both before and after gonadectomy, and this is thought to be due to a combination of skeletal resistance to androgen action and oestrogen deficiency.[8,9] After gonadectomy, lifelong sex steroid replacement is necessary for optimal bone health, but reduced BMD has been demonstrated even in those with good compliance to oestrogen therapy.[10] Previous reports have only included small numbers of subjects with BMD performed before gonadectomy, and the impact of timing of gonadectomy on bone health is not known.

2 | AIMS

We hypothesized that a delayed gonadectomy may be a relative oestrogen deficiency state, and this may have an adverse effect on BMD. We tested this hypothesis in several ways. First, a simple correlation was sought between age of gonadectomy and BMD. Second, BMD in two groups defined as early and late gonadectomy were compared. Third, in those in whom pregonadectomy BMD and hormone profiles were available tested for an association between gonadal function and serum oestradiol and BMD. Lastly, a subgroup in whom BMD measurements were available both before and after gonadectomy were assessed.

3 | METHODS

We performed a retrospective cross-sectional study of women with CAIS attending the adult DSD clinic at UCLH. Ethical approval was granted by the local research and ethics committee (Integrated Research Application System approval number 184846). The clinical diagnosis of women with CAIS was based on an unambiguous female phenotype, scant androgen-dependent body hair, absent uterus, karyotype and gonadal histology.

The records of 129 women with CAIS attending UCLH were screened of whom 113 had adequate bone density data and consented to their data being used for research. Subjects' weight and height were measured by standard balance and stadiometer. Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2). Age and presenting symptoms at diagnosis, the presence of hernia, age of gonadectomy and hormone replacement details were recorded. Adherence to medications was assessed through self-reporting. Pre-gonadectomy luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone and oestradiol levels were measured by immunoassay.

Bone density measurements were made using dual-energy X-ray absorptiometry (DXA; Hologic QDR 4500 fan beam, Hologic Inc., Waltham, MA, USA), and were obtained between lumbar levels 1-4 (L1-L4) and at the hip. The results of BMD were expressed as T-scores, defined statistically as the number of standard deviations by which a result differed from mean young women.

Most recent DXA was assessed in all subjects and, when available, the last DXA prior to gonadectomy. Not all subjects had DXA performed prior to gonadectomy, so we also included all of those that had DXA performed within a two-year period after surgery. The period of 2 years was nominated as sex steroids are unlikely to have a major effect on bone density within this timeframe.

To assess the impact of early gonadectomy on health outcomes, we divided the group into those that had gonadectomy performed before or after the age of 14. Subjects that had not undergone gonadectomy were included in the "late gonadectomy" group.

3.1 | Statistical analysis

The statistical software programme SPSS version 22 was used for analysis (SPSS Inc., Chicago, IL, USA), and a 5% level of significance was chosen. The majority of data including bone density measurements were normally distributed and are reported as mean (95% confidence interval [95% CI]). The pregonadectomy blood test data were not normally distributed and are reported as median (range). The sample size of pre- and post-gonadectomy subjects was small, so these data are reported as median (range), and nonparametric tests were used for analysis. Overall BMD scores was assessed using one sided *t* test. BMD scores in early and late gonadectomy groups were compared using univariate analysis of variance controlling for age and BMI. Fisher's exact test and Wilcoxon signed-ranks test were applied to calculate the probability for differences between groups, and the Mann-Whitney *U* test was used to test continuous variables. Spearman's correlation coefficient was used to measure the strength of association between ranked variables.

4 | RESULTS

The clinical characteristics of the 113 subjects are shown in Table 1. The majority of the cohort (55%) had presented with primary amenorrhoea, and mean (95% CI) age of diagnosis was 12 (10.5-13.6) years. Forty-nine subjects (43%) had a history of inguinal hernia, and this was noted at a mean age of 2.3 (1.5-3.1) years. Twelve subjects (11%) had a family history of CAIS. One subject (0.8%) was diagnosed following discordance between antenatal chromosomal analysis and phenotype at birth.

Mean age of gonadectomy was 14.8 (13-16.5) years and had been performed in 104 (92%) of subjects. There were 9 subjects that had chosen not to undergo gonadectomy, and their current mean age was 31 (24-39) years. Mean height was 1.71 m (1.70-1.72), and there was

TABLE 1 Characteristics of subjects' (n=113) anthropomorphic data and latest bone mineral density measurement

Age at last visit (y)	37.3 (34.8-39.8)
Weight (kg)	73.6 (70.3-77.1)
Height (m)	1.71 (1.7-1.72)
BMI (kg/m^2)	25.2 (24-26.3)
Age at diagnosis (y)	12 (10.5-13.6)
History of hernia (n [%])	49 (43%)
Age at hernia (y)	2.3 (1.5-3.1)
Age at gonadectomy (y)	14.8 (13-16.5)
Age at latest DXA	33.8 (31.4-36.3)
Latest hip T-score	−0.3 (−0.49 to −0.12)
Latest spine T-score	−1.34 (−1.55 to −1.13)

Data are represented as mean (95% confidence interval) or n (%).

no association between age of gonadectomy and final adult height (correlation coefficient=.114, *P*=.254).

Hormone replacement comprised of oral oestradiol/ethinylestradiol (51.8%), transdermal oestradiol (28.1%), testosterone alone (5.5%), selective oestrogen receptor modulator (2.7%) and no treatment (11.5%). The subjects that were receiving no treatment had either gonads in situ or were above the average age of menopause and had discontinued treatment. The equivalent daily dose of oestrogen replacement ranged from 1 to 6 mg oral oestradiol valerate. Thirteen (11.5%) of subjects were receiving testosterone replacement, and of these, seven were receiving combination oestrogen and testosterone replacement and six were receiving testosterone alone. Thirty-eight (33.6%) subjects had reported poor adherence to their prescribed medication.

Latest dual-energy X-ray absorptiometry had been performed at a mean age of 33.8 (31.4-36.2) years. The mean BMD was reduced (95% CI); T-score was −1.34 (−1.55 to −1.13; *P*<.001) at the lumbar spine and −0.3 (−0.49 to −0.12; *P*=.001) at the hip. The mean interval from gonadectomy to the most recent DXA was 18 (15.6-20.4) years. There was no correlation between age at gonadectomy and the T-score at the lumbar spine or hip (correlation coefficient=.129, *P*=.193 and .019, *P*=.852 respectively). There was no difference in BMD in those subjects that reported poor adherence to medication compared to those without reported poor adherence.

In order to assess the impact of age of gonadectomy on BMD, subjects were divided into those that had gonadectomy before (n=41) or after (n=72) the age of 14 (early and late gonadectomy groups, respectively). The late gonadectomy group included those with gonads in situ, for whom the most recent BMD was assessed. The early gonadectomy group were significantly younger than the late group at the time of DXA (28.3 vs 37.1 years, *P*<.001) with a trend to lower BMI (24.1 vs 25.9 kg/m^2, *P*=.134). There were no significant differences in BMD between the early and late group at the lumbar spine (T-score −1.45 vs −1.28, respectively, *P*=.44), or at the hip (T-score −0.4 vs −0.25, respectively, *P*=.42) even when accounting for differences in age and BMI.

4.1 | Pre-gonadectomy analysis

Pregonadectomy blood tests were available in 31 subjects, and results revealed median (range) LH 24.2 (13-59.1) IU/L, FSH 4.6 (1.1-68.9) IU/L, oestradiol 127 (44-231) pmol/L, testosterone 22.2 (8.1-43.6) nmol/L (Table 2, and Table S1 in supplementary material). There were 32 subjects that had BMD measured before or shortly after the time of gonadectomy. Of these 32 subjects, 19 had BMD measured before gonadectomy and 13 had BMD measured postoperatively, but within 2 years of surgery. Both of these groups were analysed together as a "near-gonadectomy" group. Near-gonadectomy BMD was performed at a mean (95% CI) age of 25.4 (22-28.9) years. The mean BMD was significantly reduced (95% CI) at the lumbar spine; T-score −1.05 (−1.54 to −0.57; *P*<.001), but was normal at the hip; T-score −0.04 (−0.35 to 0.28; *P*=.8). There was no relationship between pregonadectomy BMD and BMI, the presence of hernia, testosterone, oestradiol, LH or FSH levels.

There were six subjects (19%) with a pre-gonadectomy BMD below the normal range (T-score <−2.0), and these subjects were compared to those in the normal range (T-score>−2.0). This subset analysis showed that there were no significant differences in age, BMI, the presence of hernia, age at diagnosis, age at gonadectomy, FSH, oestradiol or testosterone levels between the two groups. We assessed aromatization by calculating the testosterone:oestradiol ratio, but there was no association between this and BMD, nor was there a difference between the two groups.

4.2 | Bone mineral density before and after gonadectomy

There were 12 subjects with DXA results available both before and after gonadectomy, with a median time interval of 4.3 (1.7-12.8) years between measurements. Characteristics are shown in Table 3, and the change in

TABLE 2 Characteristics of subjects pregonadectomy laboratory parameters (n=31)

Age at blood test (y)	19.7 (13.4-52.3)
LH (IU/L)	24.2 (13-59.1)
FSH (IU/L)	4.6 (1.1-68.9)
Oestradiol (pmol/L)	127 (44-231)
Testosterone (nmol/L)	22.2 (8.1-43.6)

Data are represented as median (range).

TABLE 3 Characteristics of subjects (n=12) before and after gonadectomy.

	Pregonadectomy	Postgonadectomy	*P*-value
Age (y)	25.1 (18.3-52.3)	29.6 (20.7-62.7)	.002
Weight (kg)	72.5 (55-103)	79 (53-137)	.047
BMI (kg/m^2)	25.1 (22.2-32.1)	28.4 (21.2-42.8)	.028
Spine T-score	−1.2 (−4.2-1.0)	−0.95 (−3.4-1.2)	.721
Hip T-score	−0.4 (−1.5-2.0)	−0.2 (−1.3-1.7)	.645

Data are represented median (range)

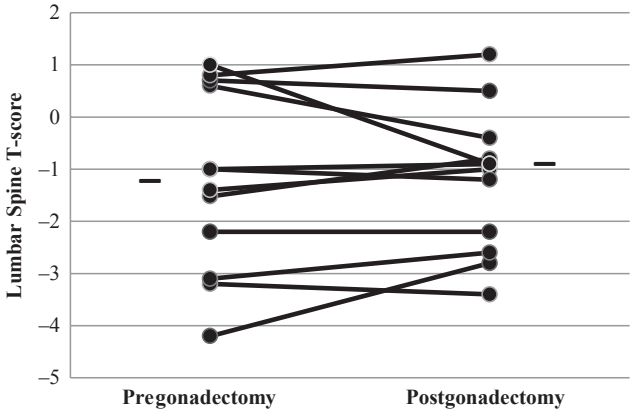

FIGURE 1 Change in bone mineral density (BMD) in subjects (n=12) before and after gonadectomy, with a median time interval of 4.3 (1.7-12.8) years. Bars represent median lumbar spine T-score before and after gonadectomy (−1.2 and −0.95, respectively, with no significant difference, *P*=.72)

lumbar spine BMD is shown in Figure 1. Oestrogen replacement had been prescribed immediately after gonadectomy in all subjects. There was a significant increase in weight and BMI (6.5 kg, *P*=.047 and 3.3 kg/m^2, *P*=.028, respectively), but there was no significant change in BMD before and after gonadectomy (T-score at lumbar spine −1.2 and −0.95, respectively, *P*=.72; T-score at hip −0.4 and −0.2, respectively, *P*=.65).

5 | DISCUSSION

In this study, we aimed to assess the impact of age of gonadectomy on bone health in CAIS, and to determine the relationship between gonadal function and BMD in order to facilitate decision making surrounding the appropriate timing of gonadectomy.

We found that BMD at both the lumbar spine and hip was significantly reduced when compared to reference values, accepting that we could not adjust for the effect of tall stature in CAIS which in any case would have reduced the BMD estimate in CAIS further. We found no correlation between age of gonadectomy and BMD; neither did we find a difference in the early vs late gonadectomy groups. This is a very reassuring finding indicating that women who choose to delay gonadectomy are not likely to jeopardize bone density.

The low BMD previously reported in subjects with CAIS is felt to be due to a combination of oestrogen deficiency and skeletal resistance to androgen action.[8] Human osteoblasts from both sexes possess 5α-reductase activity and express functional androgen receptors, so the actions of testosterone on bone in normal subjects can be mediated via testosterone directly or dihydrotestosterone (DHT) via the androgen receptor.[11] While testosterone levels in CAIS are variable, the average production has been reported to be higher when compared to males (8.3 mg/d v. 5.7 mg/d, respectively), and we report similar findings in our cohort.[12] Despite higher than normal levels of testosterone, women with CAIS are constitutionally unable to respond to androgens, and we did not find a correlation between testosterone levels and BMD in our cohort.

Testosterone is converted by aromatase to oestradiol and acts on the osteoblast indirectly via the oestrogen receptor alpha or beta.[11] When compared to those with normal ovarian function, women with CAIS and intact gonads have reduced circulating concentrations of oestrogen. The average total daily production of oestradiol has been shown to be 77 μg/d in women with CAIS compared to 90/250 μg/d in the follicular/luteal phase in normal premenopausal women, and 44 μg/d in men.[12] In males, the majority of circulating oestradiol (80%) originates from peripheral aromatization of androgen precursors, and the remainder is secreted directly from the gonads. The higher levels of oestradiol observed in CAIS compared to men may be due to the stimulatory effects of increased gonadotrophin secretion on gonadal oestradiol production in addition to extra-glandular aromatization.[8]

Oestrogen deficiency can also occur through inadequate oestrogen supplementation post-gonadectomy, and lifelong oestrogen supplementation is required after surgery. The adequacy of replacement requires careful monitoring of BMD. We assessed 12 subjects in whom we had BMD before and after gonadectomy, and we found no change in BMD after a median 4.3 years of follow-up.

Oestrogen has a well-established role in the increase and maintenance of BMD in women. Recent findings have also demonstrated a powerful and independent effect of oestrogen deficiency on bone turnover markers and BMD in men.[13] We did not find a relationship between oestradiol levels and pre-gonadectomy BMD in our cohort of women with CAIS. Similarly we did not find that gonadal failure as evidenced by an elevated FSH level had an impact on BMD. We also looked for an effect of aromatase, by calculating testosterone:oestradiol ratio, but found no correlation with BMD.

While the above observations provide theoretical links between relative oestrogen deficiency and low bone density in CAIS, we could find no statistical evidence that endogenous oestrogen status was a clinically important mechanism.

We theorized that an inguinal gonad may be vulnerable to gonadal failure. In males with cryptorchidism, testes that remain undescended are associated with progressive loss of germ and Leydig cells.[14] However, we did not find a relationship between history of inguinal hernia and FSH, LH, testosterone, oestradiol or BMD at the hip or spine. There were six subjects with low pre-gonadectomy lumbar spine BMD (T-score <−2) but there were no differences in parameters in this subgroup when compared to the rest of the subjects. This cohort requires further investigation, as it may represent a novel phenotype.

Previous studies have shown that women with CAIS are taller than average, and that adult stature in women with intact gonads approaches target male height.[15] This was borne out in our study where the mean final adult height was 171 cm, compared to the national average of 162 cm for women and 175 cm for men.[16] We did not find a correlation between age at gonadectomy and final adult height, and there was no difference in height between the early or late gonadectomy groups. We did note a significant weight gain of 6.5 kg in subjects that were followed up after gonadectomy, during a time period that coincides with the commencement of HRT. A proportion of subjects had obesity (BMI>30 kg/m^2), and the transdermal route of

oestrogen therapy may be preferable in these cases to lower the risk of thrombosis. It is also important to address cardiovascular risk factors, and subjects with CAIS should be followed up in specialized centres with experience in managing DSD.

As a retrospective analysis, our study has some inherent limitations, and the selected group of subjects that predominantly presented with primary amenorrhoea may not be fully representative of the condition. The results must be interpreted with the sample size in mind, and we cannot out rule that the lack of observed change in BMD post-gonadectomy may be due to the small number of subjects in the subgroup analyses. In addition, although the cohort attended single centre, there is heterogeneity in the group, and treatment pathways in this rare condition are individualized and may have changed during the course of follow-up. Nevertheless, a large effect of the timing of gonadectomy on BMD is not apparent in this analysis.

6 | CONCLUSIONS

We found reduced BMD at the spine and hip in subjects with CAIS. We found no relationship between age at gonadectomy and BMD, and we also found no drop in BMD in subjects followed up after gonadectomy. A subset with T-score<−2 requires further investigation as it may represent a novel phenotype. We recommend regular BMD monitoring in all subjects and lifelong sex steroid replacement after surgery. Our findings support current practice to defer gonadectomy until after completion of puberty. We could identify no long-term risk of retained gonads in terms of bone density but note the risk of malignancy in this group that cannot be attenuated by current screening tools.

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DISCLOSURE STATEMENT

The authors have nothing to disclose.

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SUPPORTING INFORMATION

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