Hormonal Contraceptive Use, Bone Density, and Biochemical Markers of Bone Metabolism in British Army Recruits

Charlotte V Coombs[1], Thomas J O'Leary[1,2], Jonathan C Y Tang[3], William D Fraser[3,4], Julie P Greeves[1,3]

[1]Army Health and Performance Research, Army Headquarters, Andover, UK; [2]Division of Surgery and Interventional Science, University College London, London, UK; [3]Norwich Medical School, University of East Anglia, Norwich, UK; [4] Depts of Endocrinology and Clinical Biochemistry, Norfolk and Norwich University Hospital, Norwich, UK.

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Corresponding Author

Julie P Greeves OBE PhD, Army Health and Performance Research, Army Headquarters, Andover, Hampshire, SP11 8HT, UK. Tel: +44 (0) 300 157 9149. Email: julie.greeves143@mod.gov.uk

Contributorship

JPG designed and conducted the study. JCYT and WDF performed the biochemical analysis. CVC performed the data analysis. CVC and TJOL produced the manuscript. All authors edited the manuscript for intellectual content and approved the final version. CVC and JPG take responsibility as guarantors for the overall manuscript content.

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ABSTRACT

Introduction: Hormonal contraceptive use might impair bone health and increase stress fracture risk by decreasing endogenous oestrogen production, a central regulator of bone metabolism. This cross-sectional study investigated bone density and biochemical markers of bone metabolism in women taking hormonal contraceptives on entry to basic military training. **Methods:** Forty-five female British Army recruits had biochemical markers of bone metabolism, areal bone mineral density (aBMD), and tibial speed of sound (tSOS) measured at the start of basic military training. Participants were compared by their method of hormonal contraception: no hormonal contraception (NONE), combined oral contraceptive pill (COCP) users, or depot-medroxyprogesterone acetate (DMPA) users (20 ± 2.8 yrs, 1.64 ± 0.63 m, 61.7 ± 6.2 kg). **Results:** aBMD was not different between groups ($p \geq 0.204$), but tSOS was higher in NONE (3%, $p = 0.014$) when compared with DMPA users. ßCTX was higher in NONE (45%, $p = 0.037$) and DMPA users (90%, $p = 0.003$) compared with COCP users. P1NP was higher in DMPA users compared with NONE (43%, $p = 0.045$) and COCP users (127%, $p = 0.001$), and higher in NONE compared with COCP users (59%, $p = 0.014$). Bone ALP was higher in DMPA users compared with COCP users (56%, $p = 0.044$). **Conclusions:** DMPA use was associated with increased bone turnover, and decreased cortical bone integrity of the tibia. Lower cortical bone integrity in DMPA users was possibly mediated by increased intracortical remodelling, but trabecular bone was not affected by contraceptive use.

Key Words: Combined Oral Contraceptive Pill, Depot-Medroxyprogesterone Acetate, Military, Stress Fracture.

Key Messages

1. Areal bone mineral density was not different between combined oral contraceptive pill users, depot-medroxyprogesterone acetate users and non-hormonal contraceptive users.
2. Combined oral contraceptive pill users had lower bone formation and resorption.
3. Depot-medroxyprogesterone acetate users had higher bone turnover, and poorer cortical bone integrity of the tibia.
4. The effect of depot-medroxyprogesterone acetate use on tibial stress fracture risk during British Army basic training should be explored further.

INTRODUCTION

Women are three times more likely to develop a stress fracture compared with men during basic military training.[1-5] This risk might further increase for women training in arduous ground close combat roles.[2-4] Stress fractures can be debilitating, require prolonged rehabilitation, and can result in medical discharge.[6] Lower areal bone mineral density (aBMD)[7] and bone speed of sound[8] are risk factors for stress fracture, although many predisposing factors are involved.[9] Strategies to protect and enhance bone health are, therefore, essential for women starting a military career.

Hormonal contraceptives are synthetic female sex steroid hormones taken to prevent pregnancy and/or control the menstrual cycle. They down-regulate the hypothalamic-pituitary-ovarian (HPO) axis thereby decreasing endogenous oestrogen production and suppressing ovulation.[10] [11] Oestrogen is a central regulator of bone metabolism and hormonal contraceptives could impair bone health.[12] [13] Hormonal contraceptives are used by servicewomen to regulate menstrual bleeding and manage symptoms during training and deployment.[14-16] The combined oral contraceptive pill (COCP) is the most common hormonal contraceptive used in the military,[15] although long-acting reversible contraception (LARC) are gaining popularity.[17-19]

The COCP decreases some markers of bone resorption and bone formation.[20-24] The evidence for the effect of the COCP on aBMD is mixed.[12] [20] [25-29] Depot-medroxyprogesterone acetate (DMPA), an injectable LARC,[30] is widely reported to impair bone health; DMPA increases bone resorption and bone formation,[23] [31-33] decreases aBMD,[12] [23] [34-36] and increases stress fracture risk in basic military training.[8] Hormonal contraceptives used by women entering basic military training may, therefore, influence stress fracture risk.

There are no data examining bone density and biochemical markers of bone metabolism in different groups of hormonal contraceptive users in the military. This preliminary study compared aBMD, bone speed of sound, biochemical markers of bone resorption and formation, and sex steroid concentrations in female recruits using either the COCP, DMPA, or no hormonal contraception at the start of British Army basic training. It was hypothesised that: 1) DMPA users would have higher bone resorption and formation, lower aBMD, and lower bone speed of sound compared with COCP users and non-hormonal contraceptive users, and 2) COCP users would have lower bone resorption and formation, but similar aBMD and bone speed of sound compared with non-hormonal contraceptive users.

MATERIALS AND METHOD

Participants

Fifty-six female standard entry British Army recruits gave voluntary written informed consent. All women were starting their 12-week basic military training course (Common Military Syllabus for Recruits) at the Army Training Centre, Pirbright. All participants had passed their military initial medical assessment and were declared medically fit to train. Participants were classified according to their method of hormonal contraception: eumenorrheic women, self-declared as menstruating regularly every 21 to 34 days, who were currently taking no hormonal contraception (NONE); COCP users, or; DMPA users. Primary or secondary functional hypothalamic amenorrhea is a bar to military service, and so our participants did not have any menstrual disturbances. The study was approved by the QinetiQ Ethics Committee (QinetiQ SP619).

Experimental overview

Participants visited the laboratory on three consecutive days. On day one after the medical assessment, lifestyle questionnaires were completed, and height and body mass were measured. On day two following an overnight fast, a venous blood sample was taken for measurement of biochemical markers of bone resorption and formation, and sex steroid concentrations. Biochemical measures were not controlled for menstrual cycle phase. On day three scans were performed for measures of aBMD and quantitative ultrasound outcomes.

Lifestyle questionnaires

Participant demographics, contraceptive use, menstrual status, and gynaecological disorders were recorded using questionnaires.

Anthropometry

Body mass was measured to the nearest 0.1 kg (Seca 770, Seca Ltd, Birmingham UK) and stature was measured to the nearest 0.5 cm (Seca 225, Seca Ltd, Birmingham UK). Tibia width was measured as the distance between the lateral and medial tibial condyles using anthropometric callipers. Tibia length was measured as the distance between the medial tibial condyle and the midpoint of the medial malleolus using a tape measure. Tibia width and length were used to calculate tibia width:length. Body fat (%) and lean mass (kg) were calculated from a whole-body Dual Energy X-Ray Absorptiometry (DXA) (Hologic QDR 4500, USA) scan with participants wearing t-shirt and shorts.

Areal bone mineral density

Whole-body, lumbar spine (L1-L4), and left total hip aBMD were measured using DXA (Hologic QDR 4500, USA). Left total hip was subdivided into the left femoral neck and left

Ward's Triangle. Areal BMD of the pelvis and left leg were derived from whole-body scans. Coefficient of variations were ≤ 2.0% for all sites.

Quantitative ultrasound

Tibia Speed of Sound (tSOS; $m \cdot s^{-1}$) was measured using the Sunlight Omnisense system™ (Tel Aviv, Israel), and calculated as the transit time of a single frequency through the outer cortex of the anterior region of the tibia. The mid diaphysis was selected as region of interest, which was determined as the mid-point between the base of the heel and the top of the knee joint when the knee was flexed at a 90° angle. All tSOS measurements were conducted on the self-reported non-dominant leg with the participant lying in a prone position. A probe was passed across the region of interest using a coupling gel applied between the transducer and the skin. The mean of the three scans is presented. Calcaneus Velocity of Sound (cVOS; $m \cdot s^{-1}$) and calcaneus Broadband Ultrasound Attenuation (cBUA; $dB \cdot Mz^{-1}$) were measured using a dry ultrasound system (CUBA Clinical™, McCue Plc, Hampshire, UK). The foot was placed in the device from a seated position, and the heel was positioned between two transducers covered with silicone pads. A coupling gel was applied to the measurement site to enhance the contact between the transducers and the skin. Participants were repositioned if measurements could not be detected by the system. All measurements were performed on the same system by the same trained operator. Previous data have reported a precision range of 0.3% to 0.94% for tSOS using the Sunlight Omnisense System[37-42], and 0.99% to 4.52%[43-46] for cBUA, and 0.44% to 0.98%[45 47 48] for cVOS using the CUBA Clinical system.

Biochemical markers of bone metabolism and reproductive hormones

Venous blood samples were drawn from the antecubital fossa into serum separator tubes and K_3 EDTA tubes (Becton Dickinson Vacutainer System, USA). Blood tubes were left to stand at room temperature for 60 mins then centrifuged at 2000 rpm at 5°C for 10 min; serum and plasma were stored at −80°C until analysis. Serum was analysed for bone-specific alkaline phosphatase (bone ALP), total 25-hydroxyvitamin D (25(OH)D), 17ß-oestradiol, follicle stimulating hormone (FSH), luteinizing hormone (LH), and sex hormone-binding globulin (SHBG). Plasma was analysed for bone resorption marker beta C-terminal telopeptide (ßCTX), bone formation marker procollagen type 1 N-terminal propeptide (P1NP) and intact parathyroid hormone (iPTH). Bone ALP was measured using MicroVue enzyme immunoassay (Quidel, Athens, OH, USA), with intra/inter-assay CVs ≤ 6.2% across the concentration range between 0.7 to 140 U/L. Total 25(OH)D was the sum of 25(OH)D3 and 25(OH)D2 measured simultaneously by liquid chromatography tandem mass spectrometry (LC-MS/MS) as described.[49] The assay was calibrated using NIST SRM972a as primary standards. The inter/intra-assay coefficient of variation (CV) was ≤ 9% across the assay measuring range, with a lower limit of quantification (LLoQ) of 0.1 $nmol \cdot L^{-1}$. 17ß-oestradiol, FSH, LH, SHBG, ßCTX, P1NP and iPTH were measured using electrochemiluminescence immunoassay (ECLIA) on the COBAS e601 analyser (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. All Roche assays performed with inter-assay imprecision CVs ≤ 4.0% across their respective measurement range.

Statistical analyses

All data was tested for normality. Differences in demographics between groups were analysed using either a one-way analysis of variance (ANOVA), or the Kruskal-Wallis test. Differences in aBMD, tSOS, cVOS, and cBUA between groups were analysed using a

one-way analysis of covariance (ANCOVA) with total 25(OH)D and lean body mass as covariates. Differences in biochemical markers of bone resorption and formation between groups were analysed using a one-way ANCOVA with total 25(OH)D as the covariate. Differences in serum hormones between groups were analysed using the Kruskal-Wallis test. Where significant main effects of group occurred, Bonferroni *post-hoc* analyses were performed to identify differences between groups. Where the Kruskal-Wallis test identified a significant main effect of group, Mann-Whitney *post-hoc* analyses were performed to identify where differences occurred with a Bonferroni correction for multiple comparisons applied. Effect sizes are presented as either eta squared (η^2), or Cohen's d_s and interpreted as either: small ($d_s = 0.2$), medium ($d_s = 0.5$), or large ($d_s = 0.8$).[50] All data were analysed using SPSS (v.25, SPSS Inc. USA) and are presented as the unadjusted mean ± standard deviation (SD), unless stated otherwise. Significance was accepted as $p \leq 0.05$.

RESULTS

Seven participants were excluded from analysis as they had recently taken the 'morning after' pill and had not since had a period, or suspected they were pregnant. Four participants declined to provide blood samples. Full data sets were available for 45 recruits (Table 1). There was no difference between groups for height, lean mass, body mass, age of menarche, and total 25(OH)D concentration. Age was significantly different between groups ($p = 0.049$), but no two groups were statistically different after Bonferroni correction.

Table 1: Participant characteristics.

	NONE (n = 25 [56%])	COCP users (n = 13 [29%])	DMPA users (n = 7 [16%])
Age (y)	19.6 ± 2.4	21.5 ± 3.2	18.3 ± 1.6
Height (m)	1.64 ± 0.07	1.62 ± 0.05	1.68 ± 0.04
Body Mass (kg)	60.7 ± 6.9	61.1 ± 6.9	66.4 ± 7.9
Lean Mass (kg)[a]	43.0 ± 5.3	43.5 ± 4.2	45.7 ± 4.4

Body Fat (%)	26.0 ± 4.0	26.7 ± 4.3	29.0 ± 2.0
Tibia Length:Width[a]	4:5	4:4	4:6
Ethnicity (%)			
Caucasian	64	85	86
Black	36	15	14
Back Squadded (%)	40	38	57
Stress Fractures (*n* [%])	3 [7%]	0 [0%]	0 [0%]
Total 25(OH)D (nmol·L^{-1})	49.8 ± 23.5	56.6 ± 23.3	33.5 ± 9.0
Vitamin D Status[b]			
Sufficient (%)	44	54	0
Insufficient (%)	24	38	71
Deficient (%)	32	8	29
Age of Menarche (y)	13.2 ± 1.2	13.6 ± 2.4	13.6 ± 1.3
1.5 Mile Run Time (s)[a]	742 ± 51	774 ± 36	752 ± 39
Duration of Contraceptive Use (Months)	Not applicable	Not fully reported[c]	9.9 ± 7.9

NONE: Eumenorrheic women, self-declared as menstruating regularly every 21- - 34 days, who were currently taking no hormonal contraceptive; COCP users: combined oral contraceptive pill users; DMPA users: depot-medroxyprogesterone acetate users.
[%] = percentage of total cohort (*n* = 45).
[a]*n* = 24 for NONE.
[b]25(OH)D (nmol·L^{-1}) categories defined as: Deficient: ≤ 30; Insufficient: 31 – 50; Sufficient > 50.
[c]Data was not fully reported in the questionnaire due to poor participant recall.

Areal bone mineral density

There were no significant differences in lean body mass ($p = 0.430$) or total 25(OH)D ($p = 0.091$) between groups. Lean body mass was a significant covariate for the lumbar spine ($p < 0.001$, $\eta^2 = 0.304$), left femoral neck ($p < 0.001$, $\eta^2 = 0.286$), Ward's Triangle ($p = 0.005$, $\eta^2 = 0.188$), left total hip ($p < 0.001$, $\eta^2 = 0.420$), whole-body ($p < 0.001$, $\eta^2 = 0.436$), pelvis ($p = 0.002$, $\eta^2 = 0.217$), and left leg ($p < 0.001$, $\eta^2 = 0.517$) aBMD; total 25(OH)D was not a significant covariate for any aBMD measure ($p \geq 0.074$). There were no significant differences in lumbar spine, left femoral neck, Ward's Triangle, left total hip, whole-body, pelvis, or left leg aBMD between groups ($p \geq 0.204$) (Figure 1). Normal aBMD *z*-scores (mean ± SD) were recorded for the lumbar spine (NONE: -0.32 ± 0.89, COCP users: -0.29 ± 1.05, DMPA users: -0.21 ± 0.87), left femoral neck (NONE: 0.01 ± 1.09, COCP users: 0.28 ± 0.80, DMPA users:

0.09 ± 1.20) and left total hip (NONE: -0.02 ± 0.99, COCP users: 0.42 ± 0.82, DMPA users: -0.03 ± 0.81) across groups.

Insert Figure 1 here

Tibia speed of sound, calcaneus velocity of sound, and calcaneus broadband ultrasound attenuation

Lean mass was not a significant covariate for cVOS (p = 0.752), cBUA (p = 0.245), or tSOS (p = 0.776); total 25(OH)D was a significant covariate for cVOS (p = 0.022, η^2 = 0.127) and tSOS (p = 0.016, η^2 = 0.140), but not cBUA (p = 0.223). There was no significant difference in cVOS (p = 0.307) or cBUA (p = 0.058) between groups. Tibial SOS was significantly different between groups (p = 0.017, η^2 = 0.189). Tibial SOS was 3% higher in NONE compared with DMPA users (p = 0.014, d_s = 1.58) (Figure 2A).

Insert Figure 2 here

Biochemical markers of bone resorption and bone formation

Total 25(OH)D was a significant covariate for P1NP (p = 0.046, η^2 = 0.094) and iPTH (p < 0.001, η^2 = 0.269), but not for bone ALP (p = 0.096) or ßCTX (p = 0.218). There was a significant difference in P1NP between groups (p = 0.003, η^2 = 0.253). P1NP was higher in NONE compared with COCP users (59%, p = 0.014, d_s = 0.94), and higher in DMPA users compared with NONE (43%, p = 0.045, d_s = 0.99), and COCP users (127%, p = 0.001, d_s = 2.70) (Figure 3A). There was a significant difference in bone ALP between groups (p = 0.030, η^2 = 0.157). Bone ALP was higher in DMPA users compared with COCP users (56%, p = 0.044, d_s = 2.05) (Figure 3B). There was a significant difference in ßCTX between

groups ($p = 0.003$, $\eta^2 = 0.249$). ßCTX was higher in NONE (45%, $p = 0.037$, $d_s = 0.99$), and DMPA users (90%, $p = 0.003$, $d_s = 2.72$) compared with COCP users (Figure 3C). There were no significant differences in iPTH between groups ($p = 0.219$) (Figure 3D).

Insert Figure 3 here

Reproductive hormones and sex hormone binding globulin

There was a significant difference in LH between groups ($p = 0.003$). Luteinizing hormone concentrations were higher in NONE (140%, $p = 0.016$) and DMPA users (264%, $p = 0.003$) compared with COCP users (Figure 4A). There was a significant difference in 17ß-oestradiol between groups ($p < 0.001$) (Figure 4B). 17ß-oestradiol concentrations were lower in COCP users compared with NONE (66 %, $p < 0.001$) and DMPA users (30%, $p = 0.014$). There was a significant difference in FSH between groups ($p = 0.002$). FSH was higher in DMPA users compared with NONE (59%, $p = 0.011$) and COCP users (206%, $p = 0.004$) (Figure 4C). There was a significant difference in SHBG between groups ($p < 0.001$). SHBG was higher in COCP users compared with NONE (166%, $p < 0.001$) and DMPA users (174%, $p = 0.002$) (Figure 4D).

Insert Figure 4 here

DISCUSSION

This study compared aBMD, bone ultrasound outcomes, and biochemical markers of bone resorption and formation between female recruits who were taking either no hormonal contraception, the COCP, or DMPA at the start of British Army basic training. Areal BMD was not different between groups, but tSOS of the cortex was lower in DMPA than and non-

users. Markers of bone resorption and formation were higher in DMPA users than COCP users and non-users after adjusting for vitamin D status, and lower in COCP users than non-users. Basic military training is physically arduous[51] and results in changes in bone resorption and/or formation, adaptations to tibial density and geometry,[52-54] and a two- to three-fold increased stress fracture risk in women compared with men.[1-5] Better evidence of the effect of hormonal contraceptive use on bone is important to optimise the bone adaptive response for women during basic training and manage stress fracture risk.

Areal bone mineral density

There were no differences in aBMD between groups, which agree with cross-sectional and longitudinal data reporting no significant decrease in hip and spine aBMD following short-term DMPA use (< 1 year) compared with COCP or non-users.[25 55 56] Despite the greatest aBMD decrease occurring in the first 12 months of DMPA use,[25 56] longer DMPA use (> 2 yrs) is required for more severe reductions in aBMD.[12 25 32 36 57 58] The average duration of DMPA use was short in our population, which may explain why no aBMD differences were observed between groups. Participants had also passed British Army selection and were presumably physically active. This may have protected aBMD and the accrual of peak bone mass with DMPA use; evidenced through normal aBMD *z*-scores. Our data also agree with other studies demonstrating no difference in aBMD between COCP users and non-users.[12 20 29 59]

Tibial speed of sound

Speed of sound is determined by cortical bone density and thickness.[60] The tibial mid diaphysis is largely comprised of cortical bone;[60] therefore, tSOS provides a measure of cortical bone integrity and an indication of bone strength.[61] In contrast, DXA provides a two-dimensional aBMD measurement of total bone density for the whole limb. The 3% lower tSOS in DMPA

users compared with COCP users suggests that DMPA results in earlier detectable changes in cortical bone that precede changes in aBMD. Further data examining tibial density, geometry, and microarchitecture in hormonal contraceptive users are warranted. Studies from US Army basic training report decreased tibial cortical volumetric bone mineral density in women, indicative of intracortical remodelling and increased susceptibility to stress fracture,[52] and an association between DMPA use, lower tSOS, and increased risk of stress fracture.[8] Tibial SOS has been reported to predict stress fracture risk independently from BMD.[62 63] Women using DMPA in this study might be at increased risk of tibial stress fracture risk due to compromised integrity of tibial cortical bone. In contrast, aBMD *z*-scores of DMPA and COCP users indicate that trabecular bone health was unaffected by contraceptive use.

Biochemical markers of bone formation and resorption

Combined oral contraceptive pill users can have a higher vitamin D concentration compared with non-hormonal contraceptive users.[64] Vitamin D and iPTH were not different between groups; vitamin D was, however, low in this study. Low vitamin D can increase iPTH[65], which might contribute to stress fracture risk. Beta C-terminal telopeptide and P1NP — reflecting type I collagen degradation and formation — were higher in DMPA users compared with COCP users and non-users. Bone ALP — indicative of osteoblast activity — was also higher in DMPA users compared with COCP users, suggesting higher bone turnover in DMPA users, consistent with other studies.[23 31-33] Procollagen type -1 Nterminal propeptide and βCTX were lower in COCP users than non-users, demonstrating lower bone turnover consistent with previous studies.[20-24] The different effects of DMPA and COCP on markers of bone metabolism could be explained by differences in ethinyl-oestradiol and progestin between contraceptives. The progestin in DMPA suppresses endogenous oestradiol by inhibition of the HPO axis.[30] 17β-oestradiol was not different between DMPA users and non-users, but menstrual cycle

phase and timing of DMPA treatment was not standardised, which caused high inter-individual variability of reproductive hormones in non-users. Depot-medroxyprogesterone acetate users did, however, have higher FSH, LH and lower SHBG, an endocrine response to low 17β-oestradiol; high FSH is a result of disinhibition of the negative feedback loop, whereas low SHBG protects circulating concentrations of free 17β-oestradiol. Low oestradiol increases bone turnover, favouring bone resorption, and results in trabecular and cortical bone.[66] High bone turnover with DMPA use was likely the result of low 17β-oestradiol, and may explain the lower tSOS. The COCP contains high doses of ethinyl-oestradiol, which inhibits the release of FSH thereby preventing follicle development and inhibits the LH surge.[10] 17β-oestradiol was lower in COCP users compared with non-users and DMPA users, a response consistent with COCP use,[33 67] but ethinyl-oestradiol was not measured. Ethinyl-oestradiol was likely high in COCP users — supported by high SHBG — resulting in low bone turnover. Combined oral contraceptive pill use might also decrease bone formation by decreasing circulating IGF.[68]

Limitations

Questionnaire responses to the brand of COCP and duration of use were not reported due to poor participant recall. Previous hormonal contraceptive use between menarche and study participation was not recorded; therefore, the data obtained may be influenced by a preceding hormonal contraceptive, rather than hormonal contraceptive use at the time of this study. Our conclusions are also limited by the small sample size and cross-sectional between-group design.

CONCLUSIONS

Depot-medroxyprogesterone acetate use was associated with increased bone resorption and formation, and decreased cortical bone integrity of the tibia, in healthy young women, possibly

mediated by increased intracortical remodelling; therefore, DMPA use during basic training might increase tibial stress fracture risk. Combined oral contraceptive pill use was associated with decreased bone formation and resorption, suggesting that COCP use in younger recruits could attenuate peak bone mass. Trabecular bone of the hip and spine was not affected by contraceptive use in this study. The longitudinal effect of hormonal contraceptive use on bone health and stress fracture risk in basic military training requires further investigation with a larger sample.

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COMPETING INTERESTS

None.

REFERENCES

1. Bulathsinhala L, Hughes JM, McKinnon CJ, et al. Risk of stress fracture varies by race/ethnic origin in a cohort study of 1.3 million US Army soldiers. *Journal of bone and mineral research* 2017;32(7):1546-53.
2. Cosman F, Ruffing J, Zion M, et al. Determinants of stress fracture risk in United States Military Academy cadets. *Bone* 2013;55(2):359-66.
3. HQ Army U. Interim Report on the Health Risks to Women in Ground Close Combat Roles 2016 [Available from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/536381/20160706_ADR006101_Report_Women_in_Combat_WEB-FINAL.PDF accessed November 2020.
4. O'Leary TJ, Wardle S, Rawcliffe A, et al. Understanding the musculoskeletal injury risk of women in combat: the effect of infantry training and sex on musculoskeletal injury incidence during British Army basic training. *BMJ Mil Health* 2020
5. Wentz L, Liu P-Y, Haymes E, et al. Females have a greater incidence of stress fractures than males in both military and athletic populations: a systemic review. *Military medicine* 2011;176(4):420-30.
6. Sharma J, Greeves JP, Byers M, et al. Musculoskeletal injuries in British Army recruits: a prospective study of diagnosis-specific incidence and rehabilitation times. *BMC musculoskeletal disorders* 2015;16(1):106.
7. Beck T, Ruff CB, Shaffer RA, et al. Stress fracture in military recruits: gender differences in muscle and bone susceptibility factors. *Bone* 2000;27(3):437-44.
8. Lappe JM, Stegman M, Recker RR. The impact of lifestyle factors on stress fractures in female Army recruits. *Osteoporosis international* 2001;12(1):35-42.
9. Friedl KE, Evans RK, MORAN DS. Stress fracture and military medical readiness: bridging basic and applied research. *Medicine & Science in Sports & Exercise* 2008;40(11):S609-S22.
10. Herrmann M, Seibel MJ. The effects of hormonal contraceptives on bone turnover markers and bone health. *Clinical endocrinology* 2010;72(5):571-83.
11. Wright KP, Johnson JV. Evaluation of extended and continuous use oral contraceptives. *Therapeutics and clinical risk management* 2008;4(5):905.
12. Berenson AB, Breitkopf CR, Grady JJ, et al. Effects of hormonal contraception on bone mineral density after 24 months of use. *Obstetrics & Gynecology* 2004;103(5):899-906.
13. Berenson AB, Radecki CM, Grady JJ, et al. A prospective, controlled study of the effects of hormonal contraception on bone mineral density. *Obstetrics & Gynecology* 2001;98(4):576-82.

14. Enewold L, Brinton LA, McGlynn KA, et al. Oral contraceptive use among women in the military and the general US population. *Journal of Women's Health* 2010;19(5):839-45.
15. Holt K, Grindlay K, Taskier M, et al. Unintended pregnancy and contraceptive use among women in the US military: a systematic literature review. *Military medicine* 2011;176(9):1056-64.
16. Powell-Dunford NC, Cuda AS, Moore JL, et al. Menstrual suppression for combat operations: advantages of oral contraceptive pills. *Women's Health Issues* 2011;21(1):86-91.
17. Chiles DP, Roberts TA, Klein DA. Initiation and continuation of long-acting reversible contraception in the United States military healthcare system. *American journal of obstetrics and gynecology* 2016;215(3):328. e1-28. e9.
18. Erickson AK, Nelson DA, Shaw JG, et al. Long-acting reversible contraceptive placement among active-duty US Army Servicewomen. *Obstetrics & Gynecology* 2017;129(5):800-09.
19. Stahlman S, Witkop CT, Clark LL, et al. Contraception among active component service women, US Armed Forces, 2012-2016. *MSMR* 2017;24(11):10-21.
20. Ackerman KE, Singhal V, Baskaran C, et al. Oestrogen replacement improves bone mineral density in oligo-amenorrhoeic athletes: a randomised clinical trial. *British journal of sports medicine* 2019;53(4):229-36.
21. Di Giovanni G, Roy BD, Gammage KL, et al. Associations of oral contraceptive use and dietary restraint with bone speed of sound and bone turnover in university-aged women. *Applied Physiology, Nutrition, and Metabolism* 2008;33(4):696-705.
22. Grinspoon S, Friedman A, Miller K, et al. Effects of a triphasic combination oral contraceptive containing norgestimate/ethinyl estradiol on biochemical markers of bone metabolism in young women with osteopenia secondary to hypothalamic amenorrhea. *The Journal of Clinical Endocrinology & Metabolism* 2003;88(8):3651-56.
23. Ott SM, Scholes D, LaCroix AZ, et al. Effects of contraceptive use on bone biochemical markers in young women. *The Journal of Clinical Endocrinology & Metabolism* 2001;86(1):179-85.
24. Wreje U, Brynhildsen J, Åberg H, et al. Collagen metabolism markers as a reflection of bone and soft tissue turnover during the menstrual cycle and oral contraceptive use. *Contraception* 2000;61(4):265-70.
25. Cromer BA, Bonny AE, Stager M, et al. Bone mineral density in adolescent females using injectable or oral contraceptives: a 24-month prospective study. *Fertility and sterility* 2008;90(6):2060-67.
26. Cromer BA, Stager M, Bonny A, et al. Depot medroxyprogesterone acetate, oral contraceptives and bone mineral density in a cohort of adolescent girls. *Journal of adolescent health* 2004;35(6):434-41.
27. Endrikat J, Mih E, Düsterberg B, et al. A 3-year double-blind, randomized, controlled study on the influence of two oral contraceptives containing either 20 μg or 30 μg ethinylestradiol in combination with levonorgestrel on bone mineral density. *Contraception* 2004;69(3):179-87.
28. Gersten J, Hsieh J, Weiss H, et al. Effect of extended 30 μg ethinyl estradiol with continuous low-dose ethinyl estradiol and cyclic 20 μg ethinyl estradiol oral contraception on adolescent bone density: a randomized trial. *Journal of Pediatric and Adolescent Gynecology* 2016;29(6):635-42.

29. Hartard M, Kleinmond C, Wiseman M, et al. Detrimental effect of oral contraceptives on parameters of bone mass and geometry in a cohort of 248 young women. *Bone* 2007;40(2):444-50.
30. Hadji P, Colli E, Regidor P-A. Bone health in estrogen-free contraception. *Osteoporosis International* 2019:1-10.
31. Quintino-Moro A, Zantut-Wittmann DE, Silva dos Santos PN, et al. Changes in calcium metabolism and bone mineral density in new users of medroxyprogesterone acetate during the first year of use. *International Journal of Gynecology & Obstetrics* 2019;147(3):319-25.
32. Shaarawy M, El-Mallah SY, Seoudi S, et al. Effects of the long-term use of depot medroxyprogesterone acetate as hormonal contraceptive on bone mineral density and biochemical markers of bone remodeling. *Contraception* 2006;74(4):297-302.
33. Walsh JS, Eastell R, Peel NF. Effects of depot medroxyprogesterone acetate on bone density and bone metabolism before and after peak bone mass: a case-control study. *The Journal of Clinical Endocrinology & Metabolism* 2008;93(4):1317-23.
34. Beksinska ME, Kleinschmidt I, Smit JA, et al. Bone mineral density in adolescents using norethisterone enanthate, depot-medroxyprogesterone acetate or combined oral contraceptives for contraception. *Contraception* 2007;75(6):438-43.
35. Johnson CC, Burkman RT, Gold MA, et al. Longitudinal study of depot medroxyprogesterone acetate (Depo-Provera®) effects on bone health in adolescents: study design, population characteristics and baseline bone mineral density. *Contraception* 2008;77(4):239-48.
36. Scholes D, LaCroix AZ, Ichikawa LE, et al. Change in bone mineral density among adolescent women using and discontinuing depot medroxyprogesterone acetate contraception. *Archives of pediatrics & adolescent medicine* 2005;159(2):139-44.
37. Knapp K, Blake G, Spector T, et al. Can the WHO definition of osteoporosis be applied to multi-site axial transmission quantitative ultrasound? *Osteoporosis international* 2004;15(5):367-74.
38. Oral A, Esmaeilzadeh S, Yalıman A, et al. The ability of calcaneal and multisite quantitative ultrasound variables in the identification of osteoporosis in women and men. *Turkish journal of physical medicine and rehabilitation* 2019;65(3):203.
39. Rebocho LM, Cardadeiro G, Zymbal V, et al. Measurement properties of radial and tibial speed of sound for screening bone fragility in 10-to 12-year-old boys and girls. *Journal of Clinical Densitometry* 2014;17(4):528-33.
40. Weiss M, Ben-Shlomo A, Hagag P, et al. Reference database for bone speed of sound measurement by a novel quantitative multi-site ultrasound device. *Osteoporosis International* 2000;11(8):688-96.
41. Williams J, Wilson C, Biassoni L, et al. Dual energy x-ray absorptiometry and quantitative ultrasound are not interchangeable in diagnosing abnormal bones. *Archives of disease in childhood* 2012;97(9):822-24.
42. Yao M, Ludwa I, Corbett L, et al. Bone speed of sound and physical activity levels of overweight and normal-weight girls and adolescents. *Pediatric Exercise Science* 2011;23(1):25-35.
43. Falcini F, Bindi G, Ermini M, et al. Comparison of quantitative calcaneal ultrasound and dual energy X-ray absorptiometry in the evaluation of osteoporotic risk in children with chronic rheumatic diseases. *Calcified Tissue International* 2000;67(1):19-23.
44. Njeh C, Hans D, Li J, et al. Comparison of six calcaneal quantitative ultrasound devices: precision and hip fracture discrimination. *Osteoporosis International* 2000;11(12):1051-62.

45. Rawal J, Eleftheriou K, Skipworth J, et al. Relationship between calcaneal quantitative ultrasound and hip dual energy X-ray absorptiometry in young healthy men. *Osteoporosis International* 2012;23(7):1947-56.
46. Tribl B, Vogelsang H, Pohanka E, et al. Broadband ultrasound attenuation of the calcaneus: A tool for assessing bone status in patients with chronic renal failure. *Acta Radiologica* 1998;39(6):637-41.
47. Chan MY, Nguyen ND, Center JR, et al. Absolute fracture-risk prediction by a combination of calcaneal quantitative ultrasound and bone mineral density. *Calcified tissue international* 2012;90(2):128-36.
48. Stewart A, Reid D. Precision of quantitative ultrasound: comparison of three commercial scanners. *Bone* 2000;27(1):139-43.
49. Tang JC, Nicholls H, Piec I, et al. Reference intervals for serum 24, 25-dihydroxyvitamin D and the ratio with 25-hydroxyvitamin D established using a newly developed LC–MS/MS method. *The Journal of nutritional biochemistry* 2017;46:21-29.
50. Cohen J. 2nd ed: Taylor & Francis Inc 1988.
51. O'Leary TJ, Saunders SC, McGuire SJ, et al. Sex Differences in Training Loads during British Army Basic Training. *Medicine and science in sports and exercise* 2018;50(12):2565.
52. Hughes JM, Gaffney-Stomberg E, Guerriere KI, et al. Changes in tibial bone microarchitecture in female recruits in response to 8 weeks of US Army Basic Combat Training. *Bone* 2018;113:9-16.
53. Izard RM, Fraser WD, Negus C, et al. Increased density and periosteal expansion of the tibia in young adult men following short-term arduous training. *Bone* 2016;88:13-19.
54. O'Leary TJ, Izard RM, Walsh NP, et al. Skeletal macro- and microstructure adaptations in men undergoing arduous military training. *Bone* 2019;125:54-60. doi: 10.1016/j.bone.2019.05.009
55. Scholes D, LaCroix AZ, Ichikawa LE, et al. The association between depot medroxyprogesterone acetate contraception and bone mineral density in adolescent women. *Contraception* 2004;69(2):99-104.
56. Scholes D, LaCroix AZ, Ichikawa LE, et al. Injectable hormone contraception and bone density: results from a prospective study. *Epidemiology* 2002:581-87.
57. Cromer BA, Blair JM, Mahan JD, et al. A prospective comparison of bone density in adolescent girls receiving depot medroxyprogesterone acetate (Depo-Provera), levonorgestrel (Norplant), or oral contraceptives. *The Journal of pediatrics* 1996;129(5):671-76.
58. Kaunitz AM, Miller PD, Rice VM, et al. Bone mineral density in women aged 25–35 years receiving depot medroxyprogesterone acetate: recovery following discontinuation. *Contraception* 2006;74(2):90-99.
59. Scholes D, Hubbard RA, Ichikawa LE, et al. Oral contraceptive use and bone density change in adolescent and young adult women: a prospective study of age, hormone dose, and discontinuation. *The Journal of Clinical Endocrinology & Metabolism* 2011;96(9):E1380-E87.
60. Prevrhal S, Fuerst T, Fan B, et al. Quantitative ultrasound of the tibia depends on both cortical density and thickness. *Osteoporosis international* 2001;12(1):28-34.
61. Chin K-Y, Ima-Nirwana S. Calcaneal quantitative ultrasound as a determinant of bone health status: what properties of bone does it reflect? *International journal of medical sciences* 2013;10(12):1778.
62. Nguyen TV, Center JR, Eisman JA. Bone mineral density-independent association of quantitative ultrasound measurements and fracture risk in women. *Osteoporosis international* 2004;15(12):942-47.

63. Wei S, Jones G, Thomson R, et al. Oral contraceptive use and bone mass in women aged 26–36 years. *Osteoporosis international* 2011;22(1):351-55.
64. Wolman R, Wyon MA, Koutedakis Y, et al. Vitamin D status in professional ballet dancers: winter vs. summer. *Journal of Science and Medicine in Sport* 2013;16(5):388-91.
65. Guillemant J, Le H-T, Maria A, et al. Wintertime vitamin D deficiency in male adolescents: effect on parathyroid function and response to vitamin D3 supplements. *Osteoporosis International* 2001;12(10):875-79.
66. Riggs BL, Khosla S, Melton III LJ. Sex steroids and the construction and conservation of the adult skeleton. *Endocrine reviews* 2002;23(3):279-302.
67. Isley MM, Kaunitz AM. Update on hormonal contraception and bone density. *Reviews in Endocrine and Metabolic Disorders* 2011;12(2):93-106.
68. Southmayd EA, De Souza MJ. A summary of the influence of exogenous estrogen administration across the lifespan on the GH/IGF-1 axis and implications for bone health. *Growth Hormone & IGF Research* 2017;32:2-13.

FIGURE LEGENDS

Figure 1: Areal bone mineral density (aBMD) of hormonal contraceptive users and non-users. NONE: Eumenorrheic women, self-declared as menstruating regularly every 21 - 34 days, who were currently taking no hormonal contraceptive; COCP: Combined oral contraceptive pill users; DMPA: Depot-medroxyprogesterone acetate users. Boxplots detail median, interquartile range, maximum and minimum values; ×: Mean.

Figure 2: Tibia Speed of Sound (tSOS) (A), calcaneus Broadband Ultrasound Attenuation (cBUA) (B) and calcaneus Velocity of Sound (cVOS) (C) of hormonal contraceptive users and non-users. NONE: Eumenorrheic women, self-declared as menstruating regularly every 21 - 34 days, who were currently taking no hormonal contraceptive; COCP: Combined oral contraceptive pill users; DMPA: Depot-medroxyprogesterone acetate users. Boxplots detail median, interquartile range, maximum and minimum values; ×: Mean. [§]Significant difference between NONE and DMPA users ($p \leq 0.05$).

Figure 3: Procollagen type 1 N-terminal propeptide (P1NP) (A), bone-specific alkaline phosphatase (bone ALP) (B), Beta c-terminal telopeptide region of type 1 collagen (ßCTX) (C), and intact parathyroid hormone (iPTH) (D) of hormonal contraceptive users and non-users. NONE: Eumenorrheic women, self-declared as menstruating regularly every 21 - 34 days, who were currently taking no hormonal contraceptive; COCP: Combined oral contraceptive pill users; DMPA: Depot-medroxyprogesterone acetate users. Boxplots detail median, interquartile range, maximum and minimum values; ×: Mean. [*]Significant difference between NONE and COCP users; [§]Significant difference between NONE and DMPA users; [†]Significant difference between COCP users and DMPA users ($p \leq 0.05$). ßCTX: NONE $n = 12$.

Figure 4: Luteinizing hormone (LH) (A), 17β-oestradiol (B), follicle stimulating hormone (FSH) (C), and sex hormone-binding globulin (SHBG) (D) of hormonal contraceptive users and non-users. NONE: Eumenorrheic women, self-declared as menstruating regularly every 21 - 34 days, who were currently taking no hormonal contraceptive; COCP: Combined oral contraceptive pill users; DMPA: Depot-medroxyprogesterone acetate users. Boxplots detail median, interquartile range, maximum and minimum values; ×: Mean. [*]Significant difference between NONE and COCP users; [§]Significant difference between NONE and DMPA users; [†]Significant difference between COCP users and DMPA users ($p \leq 0.05$); [‡]One extremely high value has been excluded from the boxplot to retain visual integrity. LH: NONE $n = 22$, COCP $n = 12$; 17β-oestradiol: NONE $n = 21$, COCP $n = 12$; FSH: NONE $n = 22$, COCP $n = 11$; SHBG: NONE $n = 22$, COCP $n = 12$.