

**BONE MINERAL DENSITY AND USE OF DEPOT
MEDROXYPROGESTERONE ACETATE (DMPA),
NORETHISTERONE ENANTHATE (NET-EN) AND COMBINED
ORAL CONTRACEPTIVES**

Malgorzata Elzbieta Beksinska

**A thesis submitted to the Faculty of Health Sciences, University of the
Witwatersrand, in fulfilment of the requirements for the degree of
Doctor of Philosophy.
Johannesburg, 2010**

Dedicated to
Immo, Alicja and Joe

DECLARATION

I, Malgorzata Beksinska declare that this thesis is my own work. It is being submitted for the degree of Doctor of Philosophy in the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

M. Beksinska

.....22nd..... day of March....., 2010.

PUBLICATION STATEMENT.

All publications from this thesis have been conceptualised and written up as first author by the candidate. The status of the publication and the contribution of other authors is detailed below. Reprints of all publications in print can be found in Appendix 8.

Role of authors in the publications:-

M E Beksinska:- Principal investigator. Developed protocol, designed tools, involved in all aspects of data collection, data analysis and took primary responsibility for the conceptualisation and write-up of all papers as first author.

Dr Jenni Smit:- Supervisor. Dr Smit advised and supported the candidate in the interpretation of the data, and inputted into and edited all publications.

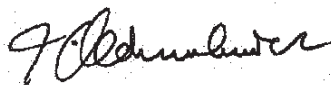
Dr Immo Kleinschmidt:- Statistician and Epidemiologist. Dr Kleinschmidt was responsible for statistical oversight. Assisted candidate in interpretation of data, complex statistical analysis, supervised candidates own analysis, and edited all the publications.

Dr Tim Farley:- Scientist, World Health Organization. Dr Farley was involved in interpretation of data, statistical advice and editing of all publications.

Prof Helen Rees:- Supervisor. Contributed to and edited two publications.

Prof F Guidozzi:- Edited and contributed to one publication.

Mrs F Mbatha:- Project coordinator of the study, edited one publication.



Dr I Kleinschmidt



Dr J Smit



Dr TMM Farley



Prof H Rees



Prof F Guidozzi



Mrs F Mbatha

PUBLICATIONS AND PRESENTATIONS

All publications have been published or are currently in press in the international Journal “Contraception”. This journal was chosen as it has historically published the key research results and extensive reviews in the area of bone mineral density and hormonal contraception. In total four papers have been published and one is in press due for publication in August 09. Further papers are submitted and in preparation for submission in 2009, related to the study objectives and other areas of data collected.

Published and in press

Beksinska ME, Kleinschmidt I, Smit JA, Farley TMM, Rees H. 2009. Bone mineral density in young women aged 19-24 after 4-5 years of exclusive and mixed use of hormonal contraception. *Contraception*, vol.80 no.2 pp.128-32.

Beksinska M.E., Kleinschmidt I., Smit J.A., Farley T.M.M. 2009. Bone Mineral Density in a cohort of adolescents during use of norethisterone enanthate, depot-medroxyprogesterone acetate or combined oral contraceptives and after discontinuation of norethisterone enanthate. *Contraception*, vol. 79, no.5 pp. 345-349.

Beksinska M, Kleinschmidt I, Smit J, Farley MM. 2007. Bone mineral density in adolescents using norethisterone enanthate, depot-medroxyprogesterone acetate, or combined oral contraceptives for contraception. *Contraception*, vol.75 no.6, pp.438-43.

Beksinska M, Smit J, Kleinschmidt I, Farley T, Mbatha F. 2005. Bone mineral density in women aged 40-49 years using depot-medroxyprogesterone acetate, norethisterone enanthate or combined oral contraceptives for contraception. *Contraception*, vol.71, no. 3, pp.170-5.

Beksinska ME, Smit J, Kleinschmidt I, Rees H, Farley TMM Guidozi F. 2003 Detection of raised FSH levels among older women using depot medroxyprogesterone acetate and norethisterone enanthate. *Contraception*, vol. 68, no. pp.339-43.

Beksinska M, Smit J, Kleinschmidt I, Milford C, Farley TMM. Prospective study of weight change in new adolescent users of DMPA, NET-EN, COCs and non-users of hormonal contraception. *Contraception*, 2009, vol 80. pp.128-132.

In preparation

Beksinska M, Smit J, Kleinschmidt I, Farley TMM. Bone mineral density in older long-term users of depot-medroxyprogesterone acetate, norethisterone enanthate or combined oral contraceptives: A longitudinal study. In preparation

Beksinska M, Smit J, Kleinschmidt I, Milford C, Farley TMM. Effects of body mass index and follicle stimulating hormone on bone mineral density in older premenopausal and perimenopausal women. In preparation

Conference Presentations

Beksinska M, Smit J, Kleinschmidt I, Rees H. Effect of injectable contraceptives on weight after one year of use. South African Society of Obstetrics and Gynaecology. Cape Town April 2003.

Beksinska M, Smit J, Kleinschmidt I, Rees H, Farley TMM, Mbatha F. Effect of injectable contraceptives on bone mineral density and weight after one year of use. 8th Reproductive Health Research Priorities Conference, Wild Coast, Eastern Cape 2002.

Beksinska ME, Smit J, Kleinschmidt I, Rees H, Farley TMM, Guidozzi F. Can Follicle Stimulating Hormone (FSH) be used to indicate menopause in older users of DMPA and NET-EN. 8th Reproductive Health Research Priorities Conference Wild Coast, Eastern Cape, 2002.

Beksinska M, Smit J, Kleinschmidt I, Rees H. Effect of injectable contraceptives on weight. 7th Reproductive Health Research Priorities Conference, Drakensburg, KwaZulu Natal, 2001.

ABSTRACT

Many studies have shown a negative effect of depot-medroxyprogesterone acetate (DMPA) hormonal contraception on bone mineral density (BMD) in women. There is limited information on the effect of norethisterone enanthate (NET-EN) on BMD and the effect of combined oral contraceptives (COCs) on BMD is inconclusive, however emerging evidence is showing that low-dose COCs maybe detrimental to BMD in young women. The aim of this research was to evaluate, in a 5-year follow-up study, the possible effect of DMPA, NET-EN and COCs on BMD among young (15-19 years) and older (40-49 years) South African women.

Method: This prospective study was conducted at the Commercial City Family Planning clinic in Durban, South Africa between 2000 and 2007. In the adolescent group women with no history of hormonal contraception who were initiating use of DMPA (n=115), NET-EN (n=115) or COCs (n=116) and 144 nonuser controls were recruited. In the older group, one hundred and twenty seven users of DMPA, 102 NET-EN users and 106 COC users of at least one year were compared to 161 nonuser controls. BMD was measured at the distal radius and midshaft of the ulna using dual x-ray absorptiometry. In the cross-sectional component of the study conducted at the end of the longitudinal phase, BMD was measured at the hip, spine and femoral neck in a sub-group of 96 of the younger women.

Results: In the longitudinal study of adolescents, BMD increased in all four groups during follow-up ($p < .001$). There was evidence for lower BMD increases per annum in

NET-EN ($p=.050$) and COC ($p=.010$) users compared to nonusers but no difference between DMPA and nonusers ($p=.76$). In 14 NET-EN discontinuers, an overall reduction of 0.61% per year BMD was followed, upon cessation, by an increase of 0.69% per year ($p=.066$). The cross-sectional sub-study found that young women in the injectables-only user group had lower BMDs compared to the non-user group after adjusting for BMI at the spine ($p=0.042$), hip ($p=0.025$), and femoral neck ($p=0.023$). The mixed COC/injectable user group BMD values were lower than controls; however, they were not significant at any of the three sites.

In the older women, there was no significant difference in radius BMD between the contraceptive user groups and the non-user controls ($p=.26$) with and without adjustment for age at baseline, or after two and a half years of follow-up ($p=0.52$).

Conclusion: This study suggests that BMD increases in adolescents may be less in NET-EN and COC users; however, recovery of BMD in NET-EN users was found in the small sample of adolescents followed post-discontinuation. The cross-sectional sub-study showed similar findings in long-term injectable users, but not when women had mixed injectable and COC use. There was no evidence that long-term use of DMPA, NET-EN and COCs affected BMD in the older women.

Acknowledgements

There are many people who assisted in the completion of this study and this thesis. Firstly I would like to thank my supervisors Dr. Jennifer Smit and Prof Helen Rees. I am indebted to Jenni for her academic input, support and most of all her daily encouragement. She has given a great deal of time including her own private time in assisting in the management of the project and giving direction in the writing of the thesis. Prof Helen Rees has been a great source of support and encouragement throughout this project both personally and at an academic level.

I would like to thank the staff members of the WHO for their support of this study; Dr Olav Meirick for his technical support during protocol development and set up of the study; Dr Tim Farley for support to the study once commenced and continuing beyond the end of the funded time period.

I would like to thank my colleagues Dr Arthi Ramkissoo, Mrs Zonke Mabude and Mrs Busi Kunene for their personal support and encouragement.

The data collection continued on a full-time basis for 6 years and required considerable staff input. I am therefore extremely grateful to the study staff. Firstly, Mrs Fikile Mbatha who was the Project Co-ordinator from the planning phase of the study until completion of follow-up. She was totally committed to ensuring the smooth day to day running of the study and constantly encouraged and motivated the study participants to continue in their follow-up. Ms Nontobeko Mdlalose who assisted with data collection and data

entry. Ms Malini Govender, responsible for double data entry and data cleaning. This study could not have been completed without their hard work.

Dr Immo Kleinschmidt who assisted with the statistical analysis from protocol development and throughout the study. He gave direction and advice on methods of analysis. Finally I would like to acknowledge his personal support, encouragement and giving up his free time to assist the write up of results of this study.

I would like to thank the Department of Health, KwaZulu-Natal for allowing the study to have a permanent base at the Commercial City Family Planning Clinic in Durban for almost 7 years. In particular, Mrs Rajees Kanjee, the clinic supervisor for supporting the study by providing a consulting room and allowing study staff to use the facilities of the clinic.

I wish to acknowledge the World Health Organisation (WHO), Department for International development (DFID) and William and Flora Hewlett Foundation for financial support of this study.

Finally I would like to thank my family:- Immo, Alicja and Joe for their support and encouragement.

TABLE OF CONTENTS

	Page
DEDICATION	i
DECLARATION	ii
PUBLICATION STATEMENT	iii
PUBLICATIONS AND PRESENTATIONS	iv
ABSTRACT	vii
ACKNOWLEDGEMENTS	ix
TABLE OF CONTANTS	xi
LIST OF TABLES	xvii
LIST OF FIGURES	xix
LIST OF ABBREVIATIONS AND ACRONYMS	xx
CHAPTER 1 INTRODUCTION	1
1.1 Background	1
1.1.1 Factors affecting bone formation	1
1.1.2 Reproductive hormones and bone mineral density	2
1.1.3 Hormonal contraceptive use and bone mineral density: an overview and statement of the problem.	3
1.2 Justification for the research	8
1.3 Aim and research questions	9
1.4 Format of the thesis	10

1.5 Contribution to the field	10
1.6 Literature review	13
1.6.1 Organisation of the literature review	13
1.6.2 Combined oral contraception	14
1.6.2.1 Background and historical perspective	14
1.6.2.2 COCs and Fracture risk	16
1.6.2.3 COCs and BMD outcome	18
1.6.3 Depot medroxyprogesterone acetate	29
1.6.3.1 Background and historical perspective	29
1.6.3.2 DMPA and Fracture risk	32
1.6.3.3 DMPA and BMD outcome	32
1.6.4 Norethisterone enanthate (NET-EN)	55
1.6.5 Other hormonal methods of contraception	56
1.6.5.1 Contraceptive implants	56
1.6.5.2 Monthly injectables	59
1.6.5.3 Progesterone only oral contraceptives	60
1.6.5.4 Progesterone vaginal rings	61
1.6.5.5 Intrauterine systems	61
1.6.6 Studies on hormonal contraception and BMD outcome: An overview of sites measured, equipment used and study limitations.	62
 CHAPTER 2 MATERIALS AND METHODS	 66
2.1 General outline	66

2.2 Recruitment procedure	67
2.3 Criteria for selection of subjects	68
2.4 Admission procedure	70
2.5 Bone mass measurements	71
2.6 Visit schedule	72
2.7 Description of the DEXA equipment for BMD measurement	72
2.8 Menopausal status of women in the 40-49 age group	74
2.9 Sample size	77
2.10 Statistical analysis	78
2.11 Data management	81
2.11.1 Data collection	81
2.11.2 Data entry	81
2.12 Duration of the study	82
2.12.1 Overview	82
2.12.2 Preparation phase	82
2.12.3 Pilot phase	82
2.12.4 Enrolment phase	82
2.13 Main problems experienced	83
2.14 Ethical considerations	83
2.15 Study procedures	84
2.15.1 General principles	84

2.15.2 Recruitment	85
2.15.2.1 15-19 age group	86
2.15.2.2 40-49 age group	87
2.15.3 Screening	87
2.15.4 Admission	87
2.15.4.1 Physical measurements	87
2.15.4.2 Forms completed and information given at admission	93
2.15.5 Follow-up	93
2.15.5.1 General outline	93
2.15.5.2 Examinations	94
2.15.5.3 Forms completed and information given at follow-up	94
2.15.6 Non-attendees	95
2.15.7 Blood test processing	95
2.15.8 Notification of results to subjects	96
2.15.8.1 Blood test results	96
2.15.8.2 Need for referral and referral criteria	96
2.15.9 Quality control	98
2.15.9.1 Bone mass measurement	98
2.15.9.2 FSH/LH measurement	98
2.15.9.3 Physical measurements	99
2.15.9.4 Data forms	99
2.15.10 Collaborators	99

CHAPTER 3 RESULTS	100
3.1 Organisation of the results	
3.2 Screening , recruitment and follow-up	103
3.2.1 Screening and recruitment	103
3.2.2 Follow-up	
3.3 Bone mineral Density in a cohort of adolescent women during use of norethisterone enanthate, depot medroxyprogesterone acetate, or combined oral contraceptives and after discontinuation of norethisterone enanthate	
3.4 Bone mineral Density in young women aged 19-24 after 4-5 years of exclusive and mixed use of hormonal contraception	
3.5 Bone mineral Density in adolescents using norethisterone enanthate, depot medroxyprogesterone acetate, or combined oral contraceptives for contraception	
3.6 Bone mineral Density in women aged 40-49 years using depot- medroxyprogesterone acetate, norethisterone enanthate or combined oral contraceptives for contraception	

3.7 Bone mineral density in older long-term users of depot-medroxyprogesterone acetate, norethisterone enanthate or combined oral contraceptives for Contraception: A longitudinal study

3.8 Detection of raised FSH levels amongst older woman using depot-medroxyprogesterone acetate and norithisterone enanthate: The relationship between BMD, hormonal contraception and FSH.

4.0 CONCLUSION AND RECOMMENDATIONS

APPENDIX 1 Consent forms and information sheets (English and Zulu)

APPENDIX 2 Questionnaires and coding information

APPENDIX 3 Information on DEXA equipment

APPENDIX 4 Ethics Approval

APPENDIX 5 Flyers and posters used in recruitment

APPENDIX 6 Referral Letters

APPENDIX 7 Collaborators

APPENDIX 8 Published papers

REFERENCES

LIST OF TABLES

Table 2.1	Menopause classification	78
Table 3.2.1	Reasons for exclusion at screening	102
Table 3.2.2	Follow-up rates at two and four years	
Table 3.2.3	Reasons for study discontinuation	
Table 3.3.1	Socio-demographic, lifestyle and reproductive characteristics of subjects aged 15-19 years by contraceptive user group, at baseline	
Table 3.3.2	Mean BMD and BMI at admission by contraceptive group	
Table 3.3.3	Factors associated with radius BMD at study visit, estimated from random linear regression model	
Table 3.4.1	Characteristics of subjects aged 19-24 years by contraceptive user group	
Table 3.4.2	Mean spine, hip and femoral neck BMD by contraceptive user group, relative to non-users	
Table 3.5.1	Socio-demographic, lifestyle and reproductive characteristics of subjects aged 15-19 years by contraceptive user group, at baseline.	
Table 3.5.2	Mean BMD and BMI at admission by contraceptive group.	
Table 3.5.3	Mean BMD and BMI by year of age in 15-19 age group at admission.	
Table 3.5.4	Radius BMD at baseline, follow-up time and incidence of method change by contraceptive group	
Table 3.5.5	Factors associated with radius BMD at study visit, estimated from random effects linear regression model	
Table 3.6.1	Socio-demographic and lifestyle characteristics of subjects in the 40-49 age range by contraceptive method use.	

Table 3.6.2	Reproductive characteristics by contraceptive method user group.
Table 3.6.3	Factors potentially associated with radius BMD (unadjusted)
Table 3.6.4	Results of multiple regression model with BMI and FSH level
Table 3.7.1	Baseline characteristics of subjects in the 40-49 year age range by contraceptive method use
Table 3.7.2	Mean radius BMD at 2.5 years by contraceptive group
Table 3.8.1	Baseline information
Table 3.8.2	Individual FSH levels of the sub-group of users of DMPA and NET-EN with raised FSH levels at recruitment.

LIST OF FIGURES

Figure 3.2.1 Number of women admitted into each study group

Figure 3.5.1 Participant contraceptive history per study group

Figure 3.8.1 Observed and fitted FSH levels by age

LIST OF ABBREVIATIONS AND ACRONYMS

BMD	Bone mineral density
BMI	Body mass index
BSAP	Bone specific alkaline phosphate
CI	Confidence interval
COC	Combined oral contraceptive
DEXA	Dual-energy x-ray absorptiometry
DMPA	Depot medroxyprogesterone acetate
DPD	Deoxypyridinoline
EDL	Essential drugs list
EE	Ethinyl estradiol
FDA	Food and Drug Administration
FSH	Follicle stimulating hormone
HIV	Human immunodeficiency virus
HRT	Hormone replacement therapy
IPC	Injectable progestogen-only contraceptive
IUD	Intrauterine device
kg	Kilogram
KZN	KwaZulu-Natal
L	Litre
LH	Lutenizing hormone
LI	Levonorgestrel implant
LNG-IUS	Levonorgestrel-releasing intrauterine system

Mcg	Microgram
mg	Milligram
ml or mL	Millilitre
MPA	Medroxyprogesterone acetate
N or n	Number; sample size
NET	Norethisterone
NET-EN	Norethisterone enanthate
OR	Odds ratio
POC	Progestogen-only contraceptives
POP	Progestogen only pills
QC	Quality control
RCT	Randomised controlled trial
RR	Relative risk
SADHS	South Africa Demographic and Health Survey
SD	Standard deviation
SEXA	Single-energy x-ray absorptiometry
STI	Sexually transmitted infection
USA	United States of America
USPSTF	United States Preventive Services Task Force
WHO	World Health Organization

CHAPTER 1 INTRODUCTION

1.1 Background and statement of the problem

1.1.1 Factors affecting bone formation

Bone mass increases rapidly from birth, and during adolescence women will gain 40-50% of their skeletal mass (Sabatier et al, 1996). Up to 90% of total adult bone content will be accumulated by the age of 20 years (Cromer and Harel, 2000). More recent data from a longitudinal study found bone mineral density plateaued by age 18. Bone strength, known as bone mineral density (BMD), can be assessed by a bone mineral density test which measures how many grams of calcium and other bone minerals are packed into a segment of bone (Hayes et al, 1991). The World Health Organization (WHO) has developed a classification system which uses the population mean as a reference against which a BMD measurement can be compared (WHO, 1994). A BMD measurement can be reported as a T-score and is expressed in standard deviations (SDs) from the normal population mean. The definition of normal BMD is no more than 1 SD below the young adult normal value. Low bone mass (osteopenia) is defined as 1 SD to 2.5 SDs below the population mean and below this level osteoporosis is diagnosed (WHO, 1994).

BMD remains fairly stable in adult premenopausal women (Dempster,1995), and although some studies report that a small percentage (<1%) of bone is lost on an annual basis in premenopausal women (Genant et al, 1988), others show there is no loss or loss is limited to some bone sites only (Sowers, and Galuska, 1993). Women lose bone mass over and above age-related bone loss in association with menopause (Duursma et al,

1991; Lindsay 1987; Hui et al, 1982). It is important to know what factors affect bone formation and resorption generally. In particular, factors affecting peak bone mass in young women and bone loss in older woman are important, as these are the chief determinants of a woman's susceptibility to osteoporosis, a major health problem in older/menopausal women, causing fractures, disability, pain and deformity (Riggs and Melton, 1986).

There are several areas of influence on bone mass including:- lifestyle factors such as diet, alcohol consumption, smoking, low body weight especially anorexia nervosa, and physical exercise (Baxter-Jones et al, 2008; 1992; Mazess and Barden, 1991; Slemenda et al, 1991; Stevenson, et al, 1989; Dalsky, 1987; Krolner et al, 1983); race, and genetic factors (Morrison et al, 1994; Sowers and Galuska 1993); the rapid growth phase during adolescence has been shown to be particularly sensitive to diet and exercise (Baxter-Jones et al, 2008; Slemenda et al, 1991); chronic diseases affecting bone metabolism and calcium metabolism are known to aggravate bone loss. Finally hormonal status, including reproductive hormones are known to affect peak bone mass, and bone maintenance (Sowers and Galuska, 1993).

1.1.2 Reproductive hormones and bone mass

Of the range of hormones that can affect bone, estrogen is a reproductive hormone that plays the most important role in BMD maintenance in women (De Cherney, 1993). Estrogen deprivation can lead to bone loss and this situation can arise in a number of ways. Estrogen production begins to decline in association with the menopause and this

decline is thought to begin as early as 40 years (Duursma, 1991; Hui et al, 1982; Lindsay, 1980). Hysterectomy has been linked to reduced blood supply to the ovaries, which in turn diminishes oestrogen production (Siddle et al, 1987). Increase in the rate of bone loss is also associated with oophorectomy (Richelson et al, 1984; Cann et al, 1980; Lindsay et al, 1980). The relationship between bone loss and lactation remains unresolved as the biological process involved in the suppression of ovarian function and lactation is not clear. Some studies report that bone mass is lost as a result of lactation (Hayslip, 1989; Chan et al, 1987), while others have shown no effect (Silverstein, 1992; Alderman, 1986). Hormone replacement therapy (HRT) also provides strong evidence for the effect of estrogen on bone loss (Munk-Jensen, et al, 1988; Ettinger et al, 1987; Lindsay et al 1984; Hammond and Maxson, 1982; Aitken, 1973). Estrogen has an anabolic effect on bone, as demonstrated by the increase in BMD measurements in post-menopausal women on HRT, suggesting there may be new bone formation as well as preservation (Munk-Jensen et al, 1988).

1.1.3 Hormonal contraceptive use and bone mass

The relationship between estrogen deficiency and bone loss suggests that hormonal contraceptive use may affect BMD. The non-contraceptive benefits of estrogen and progestin combined oral contraceptives (COCs) have included treatment of hypoestrogenic conditions in women where BMD is affected (Castelo-Branco et al, 2001; Haenggi et al, 1994; Seeman et al, 1992). Conversely, concerns have been raised regarding progestogen-only contraceptives, as the absence of estrogens from these methods and the resulting hypo-estrogenic effect could lead to suppression of bone mass

acquisition. In particular, concern is greatest in the age groups where BMD is in transition; younger women who have yet to reach peak bone mass and older women entering perimenopause and menopause where age-related bone loss has commenced.

Depot-medroxyprogesterone acetate (DMPA), marketed as Depo-Provera[®], and norethisterone enanthate (NET-EN) marketed as Nur-isterate[®] are both long-acting, synthetic, progestogen-only injectable contraceptives. In 1991, the first study showing a negative impact of DMPA on BMD in adult women users was published (Cundy et al, 1991). Although many studies about DMPA and bone health followed, due to its limited availability globally, no studies were conducted on NET-EN.

Various regulatory bodies acted swiftly in response to emerging data reporting bone loss in DMPA users. In 2004, the United States Food and Drug Administration (US FDA) required the addition of a black box to the product labeling with a warning stating that DMPA may impair BMD (United States Food and Drug Administration, 2004). New patient labeling was introduced for DMPA which states “losing calcium from your bones” is a risk of the method and indicates that long term use (for more than 2 years) increases the risk of “weakening” the bones and suggests that after two years of use the healthcare provider may suggest another method or advise a “test of the bones.” Similar recommendations were made by the UK Medicines and Healthcare Products Regulatory Agency (MHRA) (Faculty of Family Planning and Reproductive Health Care, 2004), however, neither of these regulatory bodies specified which method of contraception users should switch to after two years of DMPA use.

The third edition of the World Health Organization (WHO) Medical Eligibility Criteria for contraceptive Use (WHO, 2004) changed the classification of use of DMPA and NET-EN for women below 18 years and above 45 years from category 1 which states that there is no restriction to the use of the contraceptive method, to category 2 where the advantages of using the method generally outweigh the theoretical or proven risks. Although there was an absence of evidence for NET-EN similar recommendations were applied.

In 2005, shortly after the changes in regulatory recommendations, the WHO convened an expert consultation to assess current evidence on the relationship between the use of all available steroid hormonal contraceptives and bone health. A statement on hormonal contraception and bone health (WHO, 2006) followed the meeting concluding that adolescent COC users may gain less BMD compared to non-users of the same age but not of clinical significance, whilst perimenopausal COC users may benefit from some gain in BMD compared to non-users, however findings were inconsistent. Data on the other combined hormonal contraceptives (i.e. containing estrogen and progestogens), such as the combined injectables, vaginal rings and skin patches was too limited to review. Data on progestogen-only methods showed that levonorgestrel implants did not appear to have any effect on BMD.

With regard to the progestogen injectables, both DMPA and NET-EN were considered at the meeting. The data reviewed indicated that DMPA reduced BMD in women who had

attained peak bone mass and impaired the acquisition of BMD in those who have not yet attained peak bone mass (adolescents and young women). For DMPA, the magnitude of effect seen in cross-sectional studies of long-term users showed an approximate reduction of BMD of 0.5 SD at the hip and spine compared to non-users. In longitudinal studies of adults and adolescents around 5-7% of bone was reported to be lost (approximately 0.5 SD at hip and spine after two years of use) however, the rate of loss appeared to decrease over time. On discontinuation of DMPA, BMD increased regardless of age, and within a two-to three year period BMD recovered to the level of a comparable non-DMPA user population. However, BMD did not recover in DMPA users who had reached menopause. The consultation agreed that there was not enough data to indicate whether women who had yet to reach peak bone mass would be affected i.e. whether the recovery seen in adult women could in fact apply to adolescents and young women. In addition, woman using these methods during the menopausal transition who would be losing bone for age related reasons could also be at risk, in that they would not have had a period of recovery before they enter menopause. The consultation stated that there was not enough evidence to assess whether DMPA use modified fracture risk of users in the long term. However, since the effect of DMPA on BMD was reversible, any lifetime increase in fracture risk was likely to be small. Again, in the continued absence of any evidence for NET-EN the consultation applied similar recommendations made for DMPA to use of NET-EN (WHO 2006).

In South Africa, the manufacturers of both the locally used hormonal injectable contraceptives (DMPA and NET-EN) were requested, in October 2005, to provide all

available evidence to the National Department of Health on how their methods affect BMD (DoH, personal communication, 2005). However, no changes have been made to prescribing injectable contraceptives to adults or adolescents, in regard to BMD in South Africa.

There was some concern that these new recommendations could cause negative publicity and that women of certain age groups may be cautioned against using DMPA and other progestogen-only methods. A position paper published in 2006 by the Society of Adolescent Medicine (Society of Adolescent Medicine, 2006) which supported the WHO position on DMPA, stated clearly that with existing evidence, the advantages of using DMPA in women below 18 years outweigh the disadvantages. The lack of evidence on the consequences of long-term use of DMPA was acknowledged and until data became available it was recommended that prescribing DMPA to adolescents should be considered on a case by case basis and that practitioners should continue to prescribe DMPA to adolescents who have none of the listed risk factors.. The debate on DMPA and its effect on BMD, and restrictions and concerns around use especially in adolescents, have been published in many reviews and opinion pieces (Guilbert et al, 2009; Cromer, 2003; Westhoff, 2003; Bachrach et al, 2000; Kass-Wolff, 2000; Davis, 1996). The focus has been on the need to balance effective pregnancy prevention against concerns for future bone health.

The question of change in contraceptive method after two years of DMPA use has raised fresh concerns. Recent studies have reported that COCs with very low doses of estrogen

(ethinyl estradiol) may no longer provide the once assumed benefit of BMD maintenance, but in addition some studies have reported that BMD is lower in adolescent and young women users of low-dose COCs (Martins et al, 2006). Some researchers have cautioned against the 2-year recommendation which may lead to DMPA users switching to a low-dose COC product (Berenson et al, 2008; Albertazzi et al 2006) as their data have shown that women using low-dose COCs after DMPA may be particularly at risk to loss of BMD.

1.2 Justification for the research

In South Africa hormonal contraceptive use is high as reported in the South African Demographic and Health survey (SADHS) of 2003 with approximately 58.5% of Black African women practicing contraception, using either DMPA or NET-EN and 9.9% using COCs (Department of Health, South Africa, 2006). Of the two available injections, older women (>40 years) almost exclusively use DMPA (81%) compared to NET-EN (19%). These highly effective methods of contraception may be the method of choice for many women over 40 who have finished childbearing and are concerned about avoiding pregnancy. Hormonal injectables are not generally recommended in perimenopausal women where use of these methods is viewed as “contraceptive overkill” (Guillebaud, 2001). However in South Africa alternative methods suitable for older women such as sterilization and the IUD are not readily accessible in the public health sector. Women will often continue to use hormonal injectables into their late forties and beyond menopause as menopausal symptoms such as amenorrhea may be masked by use of

progestogen-only hormonal contraceptives which also cause amenorrhea (Guillebaud, 2001).

As menopause is known to be associated with bone loss (Duursma, 1991; Lindsay 1987; Hui et al, 1982), it is important to understand more about whether using certain hormonal methods of contraception could further contribute to bone loss in this group of older South African women.

In the case of younger women, NET-EN is the more commonly used hormonal injectable (Department of Health, South Africa, 2006), with over half of young sexually active women African women aged 15-19 years using this method, fewer use DMPA (12%) and less COCs (7%). At the start of the study reported in this thesis there was limited data to indicate whether women who have yet to reach peak bone mass would recover BMD on discontinuation from DMPA, and there was no information on NET-EN use and its effect on BMD. Almost all studies on the effect of hormonal contraception and BMD have been conducted in the US and Europe, thus, in South Africa specifically and internationally there is a need for more information.

1.3 Aim and research questions

The aim of the research was to evaluate, in a prospective study, the possible effect of commonly used progestogen-only injectable contraceptives (DMPA and NET-EN) and COCs on BMD among young (15-19 years) and older (40-49 years) women.¹

The main research question was to determine if long term use of hormonal contraceptives (COCs, DMPA and NET-EN) compared to non-use is associated with an increase, decrease or no change in bone mass in women aged 15-19 or 40-49 years. A secondary objective was to investigate the relationship between hormonal injectable use and follicle stimulating hormone (FSH) levels to see if FSH could be used as an indicator of menopausal status in older injectable users. This was due to the high prevalence of amenorrhea among injectable users, which made it impossible to use the menstrual cycle as an indicator of menopausal status.

1.4 Format of the thesis

This thesis is divided into 4 Chapters as follows: 1.Introduction including the literature review; 2. Methodology; 3. Results in the form of published papers and papers in print and preparation and 4. Conclusion which briefly summarises key findings in the context of the published literature, looks at implications of the results and makes recommendations for future work. The published papers contain their own methodology however, chapter 2 provides detailed aspects of methodology and study procedures.

¹ Footnote: Other hormonal methods were not included as they are not widely used and not available in the South African public health sector

1.5 Contribution to the field

Although there is a growing body of evidence on the effect of hormonal contraceptives on BMD, this was the first study to date to investigate the effect of NET-EN on BMD, as this contraceptive method has not been widely used outside South Africa. NET-EN is increasing in popularity in this country (Department of Health South Africa, 2006) and in other countries. Therefore this work will inform national and international organizations' and regulatory bodies' guidelines and recommendations. In addition there is limited information regarding BMD and DMPA and COC use in African women. Finally, a unique aspect of this study was to investigate BMD in young women who have a history of mixed hormonal contraceptive use.

Published findings from this thesis on the baseline data from the older users (Beksinska et al, 2005) have been cited 11 times in other peer-reviewed journals. As the first study examining NET-EN and BMD it was included in two comprehensive international reviews examining progestogen-only contraception and bone mineral density (Curtis et al, 2006), and COCs and bone health (Martins et al, 2006). The cross-sectional baseline component of this study (longitudinal data was not available at the time from the study) was rated and graded as II-3, Fair, in these reviews using the system developed by the United States Preventive Services Task Force (USPSTF) (Harris et al, 2001) and was one of only 21 from 167 available studies considered for inclusion in the review. This article is also cited in a Cochrane review (Lopez et al, 2006). Preliminary analysis of this study was made available to the WHO review of bone health in 2005 (described earlier; p.5).

Two published papers have reported on the longitudinal findings from the younger age group, the first, an interim analysis conducted after 2 and a half years (Beksinska et al, 2007) has been cited 5 times. On completion of the follow-up a second paper including information on discontinuation has been published recently (Beksinska et al, 2009). A further paper due for publication in August 09, is a cross-sectional investigation of BMD in the spine, hip and neck in the younger age group at the end of the five-year follow-up period and is the first study to investigate the effect of long term mixed hormonal contraceptive use on BMD (Beksinska et al, 2009 in press).

The sub-study which examines BMD and FSH levels in DMPA and NET-EN users (Beksinska et al, 2003) has been cited 6 times and has been used by the Faculty of Family Planning and Reproductive Health Care (FFPRHC) UK to provide international guidance for contraception in women aged over 40 (Journal of Family Planning and Reproductive Health Care, 2005). This finding has important implications for guiding family planning providers in the management of older women using hormonal injectable contraception. The information published from this study has been submitted to and discussed with the South African National Department of Health. The national contraceptive guidelines are currently under revision and the information arising from this work will be included as a recommendation in the revised guidelines. The information from this study has also contributed to the *Global family planning handbook. Family Planning: A Global Handbook for Providers* (WHO, 2007).

Further publications will include a review of the status of BMD and hormonal contraception in adolescents, based on the literature review. Data analysis continues and papers are in preparation on other areas of data collected during the course of the study including weight change in long-term contraceptive users and the relationship between FSH and BMD.

1.6 Literature review

1.6.1 Organisation of the literature review

This literature review will focus on the relationship between hormonal contraceptive methods used in this study (DMPA, NET-EN and COCs) and BMD. Other hormonal contraceptive methods and their reported relationship with BMD are documented in less detail in section 1.6.5

There are over 170 articles published that have contributed research results on progestogen-only hormonal contraception and bone mineral density (Curtis and Martins, 2006), and almost 1000 on combined hormonal contraception and bone health (Martins et al, 2006). Available data on the effect of steroid hormone contraceptive use on BMD is from studies on combined oral contraceptives, progesterone-only oral contraceptives, progestogen-only injectable contraceptives, progesterone vaginal rings, combined monthly injectables, levonorgestrel implants and intrauterine systems. In the progesterone-only injectable group there is a large body of information on the 3-monthly depot medroxyprogesterone acetate (DMPA), but limited information on the 2-monthly norethisterone enanthate (NET-EN) until information was published from this thesis (Beksinska et al, 2005) and another South African study (Rosenburg et al, 2007).

For each hormonal contraceptive method, the literature on the effect of the hormonal method on fracture risk is first reviewed, followed by the relationship of the hormonal method with BMD outcome. This thesis did not investigate if there was any relationship between fracture risk and hormonal contraception and there is generally very limited data available in this area. However, as fracture is an important outcome, available information is included. For BMD outcome the review has been divided into three broad age categories 1. Adult premenopausal women 2. Adolescents and young women 3. Older women (peri and post menopausal). Within these age categories both longitudinal (prospective) and cross-sectional studies are reviewed. The review will begin with combined oral contraception and its relationship with bone health as historically the link between use of hormonal contraception and BMD developed from work in this area. A review of DMPA will follow and then the third method investigated in this thesis NET-EN will be reviewed. The hormonal methods not included in this study will be discussed in the final part of the literature review.

This literature review has attempted to limit discussion in each section to one hormonal method. Where studies reviewed include other methods, the results of the other methods are mentioned only briefly. More information is given in the section dealing with that particular method. Although this means that some studies may be reported in two sections, repetition has been kept to a minimum. The alternative would have been to discuss studies together which would have made the literature review difficult to follow.

1.6.2 Combined oral contraception

1.6. 2.1 Background and historical perspective

Combined oral contraceptives are made up of two hormones- a progestogen and an estrogen. Their action to prevent pregnancy is primarily by preventing the release of eggs from the ovaries. Prior to the first report suggesting a relationship between BMD and hormonal contraception in humans, a number of studies reported lowered serum and urinary concentrations of magnesium, calcium and phosphorous after relatively short-term use of a number of contraceptive steroids (Simpson, 1972; Goldsmith, 1966). These values remained low even after prolonged use of the method (Simpson, 1972). Further, BMD in female rats was found to increase when they were treated with Envoid, an oral contraceptive (Goldsmith, 1967). It was then postulated that COCs would have a similar effect in women. The early body of work on the effect of steroid contraceptive use and BMD was initially limited to the COCs with one of the first studies published in 1975 (Goldsmith and Johnston, 1975) indicating that COCs containing oestrogen (100mcg mestranol) showed an increase in BMD at the distal radius compared to non-user controls.

Numerous studies to date have investigated the association between COC use and BMD. Most of these studies have used BMD as an outcome, with fracture risk outcome reported far less frequently. Early studies conducted were mainly cross-sectional and in most cases the COC formulation was not reported. Since the introduction of COCs in the early 60's there has been a gradual reduction in the dosage of steroids. The relationship between high estrogen doses and thrombotic risk led to recommendations by the USFDA that the

dose of the estrogenic component of oral contraceptives be as low as possible. The phasing in of lower dose oral contraceptives that are equal to or less than 30mcg/day ethinyl estradiol (EE) has addressed this health issue, however the once assumed benefits of BMD maintenance now requires review and may be lost at these low estrogen levels.

Unlike some progestogen-only methods of contraception such as DMPA and NET-EN which have not changed in formulation, the changes in dose of EE in COCs over time has made it more difficult to compare results from earlier published studies where EE content of COCs may have been much higher. The literature review for the relationship between COCs and BMD will therefore focus on more recent studies, and also more importantly, where the COC formulation is reported. Cohort studies published since the late 90s have been more likely to specify the formulations whilst many cross-sectional studies have collected data from women who may not have known the formulation of the COCs they have used. This is obviously a particular problem when collecting data from women who have had a long history of COC use. In addition, long term users may have changed COC brand and started with a higher dose COC in their early contraceptive history but over the years this may have changed. It is still important to examine some of the early work because the general consensus of effect of COCs on BMD from these early studies was considerably more positive. There is a need to re-evaluate the implications for BMD as EE doses have come down.

1.6.2.2 COCs and Fracture risk

The first study investigating COC use and fracture risk (Cooper et al, 1993) was a large prospective cohort of women aged between 25 and 65 years which contributed 484,083 women years of follow-up. The group of ever-users of COCs (n=284,882) were more likely to have a fracture than never users (Relative Risk=1.2, 95% CI 1.08-1.34). There was however no effect of duration of use on fracture risk and COC dose was not specified. Two further large prospective cohorts (Barad et al, 2005; Vessey et al, 1998) also found an increase in fracture at all sites in COC users. Vessey et al, 1998 found an increase in fracture risk with duration of use of COCs in 187,000 COC users aged between 25 and 39 years compared to 123,00 non-users. More recently (Barad et al, 2005) found that postmenopausal women using COCs for over five years had no increased risk of fracture whilst those who used COCs for a shorter duration (5 years or less) had an increased risk of fracture. Neither of these studies specified COC formulation.

Three large cross-sectional studies (Johansson and Mellstrom, 1996; O'Neill et al, 1996; Tuppurainen et al, 1994) have reported on fracture outcomes. Tuppurainen et al, (1994) found no difference in fracture rates between perimenopausal ever users and never users of COCs regardless of duration of COC use. O'Neill (1996) found a protective effect of COC use in prevalence of vertebral deformity in women aged 50-79 years. Johansson et al (1996) also found a lowered risk of repeat fracture in 1489 COC users compared to 1723 never users. These women were part of a European birth cohort with a mean age of 51.2 years, however there was no detail on the relationship between COC use and initial fracture. Some studies have used a case control design to see if ever use of COCs is

different in women experiencing fracture at the hip (Michaelsson et al, 2001; La Vecchia et al, 1999; Michaelsson et al 1999,) and fracture at the forearm (Mallmin, 1994). Michaelsson et al, (2001) and Michaelsson et al, (1999) found that ever-users of COCs were 25% less likely to have hip fracture compared to never users with the higher dose COCs providing even further protection against hip fracture. This was the only study of fracture outcome that specified COC formulation, however there were no exclusive low-dose COC users. La Vecchia et al, (1999) found no difference in prevalence of hip fracture between ever users and never users, however the prevalence of COC use in the sample was low. Mallmin (1994) found no difference in distal forearm fracture rates in 301 cases and 301 controls. A recent Cochrane review (Lopez et al, 2006) found no COC randomised controlled trials (RCTs) conducted to date that have had fracture as an outcome.

In summary the fracture evidence is inconclusive. The estrogen formulation of the COCs was not available except for one study and some studies had not adjusted for variables linked to fracture risk. The limitations of the fracture evidence are discussed in more detail in a systematic review of combined hormonal contraception and bone health (Curtis et al, 2006) in which studies were graded using the system developed by the United States Preventive Services Task Force (USPSTF) (Harris et al, 2001). Studies with fracture as an outcome were considered to be fair with some poor, no studies were graded as good.

1.6.2.3 COCs and BMD outcome

The first major review of COC use and BMD outcome was published in 1993 (Mehta, 1993). This review concluded that COC use and its relationship with bone density was not conclusive. While seven of these early studies reported a strong protective effect (Fortney et al, 1994; Kleerkoper et al, 1991; Stevenson et al, 1989; Enzelsberger et al, 1988; Lindsay et al, 1986; Kanders et al, 1984; Goldsmith and Baumberger, 1967), six found no association between oral contraceptive use and bone mineral density (Mazess et al, 1991; Rodin et al, 1991; Lloyd et al, 1989; Stevenson et al, 1989; Hreshchshyn et al, 1988; Lindsay et al, 1986). No study was found to show a decrease in BMD. All but one (Mazess et al, 1991) of the studies included were cross-sectional. The review also identified that although COCs and BMD were being investigated there was a lack of information on other hormonal contraceptives, with the review only citing one published study on DMPA (Cundy et al, 1991) which will be discussed later.

The lack of consensus on the effect of COCs at this time can be attributed to a number of study design issues including small numbers of women and many studies not adequately controlling for factors affecting the process of bone loss. The age groups of women also varied widely with some including women across wide age ranges of 15-91 years (Kleerekoper et al, 1991), women only in their reproductive age (15-49 years), and narrow age ranges (10 years) (Lindsay et al, 1980). Composition of the brands of COCs were either not mentioned or varied in terms of the dosage of EE. Studies on COC's and BMD are described in more detail below according to age group studied.

Use of COCs and BMD outcome in adult premenopausal women

Cross-sectional studies

Numerous cross-sectional studies including premenopausal women have generally found no difference between COC users and controls (Afghani et al, 2004; Hawker et al, 2002; Ott et al, 2001; Perrotti et al, 2001; Prior et al 2001; Pascoe et al, 2000; Pettitti et al 2000; MacDougall et al, 1999; Hartard et al, 1997; Garner et al, 1995; Fortney et al, 1994; Hansen, 1994; Melton et al, 1993; Murphy et al, 1993; Kleerekoper et al, 1991; Laitinen et al, 1991; Nelson et al, 1991; Rodin et al, 1991; Hall et al, 1990; Lloyd et al, 1989; Collins et al, 1988; Hreshchyshyn et al, 1988; Picard et al, 1988; Lindsey et al, 1986; Sowers et al, 1985; Kanders et al, 1984; Goldsmith et al, 1975). In a recent systematic review of COC use and bone health (Martins et al, 2006) BMD Z scores in cross-sectional studies were graphed by age group, site of measurement and duration of COC use. The Z scores fell within the 1SD of the reference mean for almost all studies, and suggested no differences in BMD between COC users and non-users.

Prospective studies

Prospective studies have included randomized controlled trials (RCTs) and cohorts of current COC users. Nappi et al (2003) conducted a randomized trial where women were allocated to either low-dose, ultra low dose or non user groups. After one year of follow-up there was no difference between user groups and controls in spine BMD. The samples in each group (n=20) were small, however retention was high. A further RCT conducted by the same group (Nappi et al, 2005) randomized women to two types of 30 mcg EE COCs. Spinal BMD increased slightly compared to non-user controls but this was not significant. Another cohort with partial randomization (Berenson et al 2001; Berenson et

al, 2004) followed up women who were randomized to two types of COC (30ug EE/0.15 mg desogestrel or 35mcg EE/1.0mg norethindrone). These two groups were compared to DMPA users and non-hormonal user controls. After 12 months the BMD in both COC groups increased compared to DMPA and controls. After 24 months, desogestrel COC users experienced a loss of 2.6% and DMPA users had an average BMD loss of 5.7%, however this was not significant from the controls and no difference was found between either COC users groups and controls at 24 months. Another RCT that compared two COC formulations but had no non user controls (Endrikat et al, 2004) found no difference between a 20mcg EE and a 30mcg EE COC after 3 years of use.

Cohorts that have followed up new and continuing users have also found few differences between COC users and controls (Elgan et al, 2004; Paoletti et al, 2004; Elgan et al, 2003; Reed et al, 2003; Cobb et al, 2002; Weaver et al, 2001; Mais et al, 1993; Recker et al, 1992; Mazess et al, 1991). Most of these studies measured BMD at the spine and other central sites except for three studies (Elgan et al, 2004; Paoletti et al, 2004; Elgan et al, 2003) where BMD was measured at the heel.

A recent study (Gargano et al, 2008) looked at both BMD and markers for bone turnover (urinary pyridinoline and deoxypyridinoline) in young post adolescent woman using low-dose (20 & 30 mcg EE) COCs for 12 months. Although bone turnover increased significantly in the two user groups compared to controls, there was no change in BMD over the 12 months. Another recent study using investigating very low-dose (20mcg) EE COCs (Berenson et al, 2008) found that BMD was lost compared to non-users after 3

years of COC use. The loss was small and much lower than another study group of DMPA users. Women who discontinued DMPA and commenced COCs recovered BMD in the spine but not the femoral neck. The authors concluded that very low-dose COCs may result in minimal bone loss and in addition prevent recovery in those that move from DMPA to COCs. This is discussed further in the DMPA and BMD literature.

In summary, COC use and BMD outcome in adult current users in both prospective and cross-sectional studies have generally found few differences between current users and non-users. Emerging evidence is pointing towards a small loss of BMD in some studies of very low-dose COCs

Use of COCs and BMD outcome in Adolescents and young women

A number of studies have focused on adolescent users of COCs (Cromer et al, 2008; Hartard et al, 2007; Hartard et al, 2006; Lara-Torre et al, 2004; Cromer et al, 2004; Lloyd et al 2004; Rome et al, 2004; Tharnprisan et al, 2002; Lloyd et al, 2000; Cromer, 1996; Polatti et al 1995). The majority of studies have been longitudinal, with three cross-sectional studies (Hartard et al, 2007; Lloyd et al 2004; Lloyd et al 2000).

Cross-sectional studies

Lloyd et al, (2000, 2004) compared COC users and never users of COCs in a cross-sectional analyses as part of a longitudinal eight year observational study of adolescents followed-up between the ages of 12 and 20. In total 28 subjects had used COCs for at least 6 months by the age of 20 years. No difference was found in total body and hip

BMD in the two groups. Although it was known the COC users had used low-dose monophasic COCs, the actual dose of EE or type and amount of progestin was not reported and only one-third (30%) of women had commenced COCs before the age of 18.

A recent cross-sectional study (Hartard et al, 2007) of young women aged 18-24 years included 248 users of monophasic COCs, 47 never users and 201 ever users of COCs. The users were further subdivided into sub-groups depending on length of use and age of initiation. Measurements were taken in the lumbar spine, femoral neck and tibia. Results showed that women who had ever used COCs had significantly lower BMD at the femoral neck and tibial shaft compared to never users. Within the sub-groups increasing duration of use and earlier start of COCs increased risk of lower BMD in the femoral neck and lower bone mineral content (BMC) in the distal tibia. Women in the group with greater than 2 years COC use and initiation within 3 years of menarche were most compromised with a 10% reduction of BMD at the femoral neck and a non-significant 5% reduction at the spine compared to controls. Although the study stated that these women were low-dose COC users, there was no information on EE formulation, only those women using COCs with EE >50mcg were excluded.

Prospective studies

The earliest prospective study including adolescent and young women (aged 12-21) users of COCs, DMPA, Norplant, and non-user controls was published in 1996 (Cromer et al, 1996). This study found an increase of 1.5% in lumbar spine BMD at one year in COC users, although the increase in controls was greater (2.9%), this difference was not

statistically significant. This study continued to a two-year follow-up, but none of the nine COC users at year one follow-up, had continued using the method.

In further work, Cromer et al (2004) conducted a two-year follow-up in a much larger sample of 370 adolescents aged 12-18 who self selected for either a low dose (20mcg EE) COC (n=165), DMPA (n=53), or controls who received no hormonal method (n=152). At the 12-month follow-up, the mean BMD of the COC group increased by 2.3% compared to 3.8% in the control group in the lumbar spine. In the femoral neck only a small increase of 0.3% was observed in the COC group compared to 2.3% in the control group. In addition, the mean percent change in the low-dose COC group although not negative was significantly lower than the controls. The authors concluded that a COC containing 20 mcg EE/100 mg levonorgestrel could have an adverse effect on bone health in adolescents. The DMPA group showed decreases in BMD at both sites and is discussed later in this chapter. At the end of the two- year follow-up in the same study (Cromer et al, 2008) the COC group showed a mean BMD increase of 3.0 % in the neck and 4.2% in the spine. Controls were similar in the neck (+3.8%) but higher in the spine (+6.3%). In this group of adolescent girls, the bone biochemical markers serum bone specific alkaline phosphatase (BSAP) and urinary deoxypyridinoline (DPD) were measured and BSAP was found to be significantly higher in the control group compared to the COC and DMPA group (Rome et al, 2004). There was a non-significant trend towards higher DPD levels in the COC and DMPA group compared to controls. Over a 12 month period there was evidence of increased bone formation and resorption in the control group when

compared to that in the DMPA and COC groups. There was no relationship between the biochemical markers and BMD at the lumbar spine or the femoral neck.

Lara-Torre et al (2004) followed up new users of COCs and DMPA aged between 11 and 19 years and compared them to non-users. Women were followed for two years and although 71 women commenced participation in the COC group at baseline only 16 women completed one year of follow-up, and fewer (n=5), two years of follow-up. No statistically significant difference was found between COC users and controls at one and two years, however by 24 months there was a reduction of 1% in BMD in the lumbar spine among COC users compared to an increase in controls of almost 6%. At all follow-up points the COC users had higher BMD values than DMPA users and this was significant from 12 months. This longitudinal study was particularly compromised by attrition which was greatest in the COC group. The COC formulation was not specified and the study was classified as II-2, poor in the recent systematic review by Martins et al, (2006).

Polatti et al, (1995) randomized young women aged 19-22 years to receive either a low-dose COC (EE 20 mcg, desogestrel 0.150mg) or no treatment. After five years the treatment group showed no change in lumbar spine BMD from baseline whilst the control groups showed a significant increase of 7.8%. It was concluded that the COCs, although not decreasing BMD, was preventing the natural increase in BMD expected in this age group.

In a partly randomized cohort (Hartard et al, 2006) 52 women were randomized into either into a 20 mcg EE and 100mg levonorgestrel COC group or a 150 mg desogestrel COC group. After 12 months women in the desogestrel group lost 1.5% BMD at the spine while no changes in BMD were found in the spine of levonorgestrel group. In a recent study (Pikkarainen et al, 2008), bone mineral content (BMC) was measured in the spine and neck of 122 adolescent woman aged 19-22 in a four-year follow-up. Results showed a trend for a lower increase in BMC in the woman who had used COCs of ≤ 35 mcg for more than two years compared to non-users and those with 1-2 years use.

In summary, adolescents and young women should show increases in BMD over time, however the longitudinal studies discussed in this section have mainly found that although BMD does not decrease in COC users, the comparison control groups show marked increases in BMD. Some of these studies have, in addition to controls, included DMPA users, and COC users generally have higher BMDs in comparison to these users. Lifestyle and relationship factors in adolescents and young women result in high discontinuation rates and so compromise studies in being able to show significant changes over time with very reduced sample sizes. The current literature appears to point towards low-dose COC preparations compromising active bone growth, in that BMD values appear to remain static. There is limited data available on recovery in adolescent users mainly due to methodology, since those that discontinue from the method are not usually followed-up.

Use of COCs and BMD outcome in older women (perimenopausal and postmenopausal)

Perimenopausal women

The number of women using COCs in the perimenopause is limited. One cross-sectional study (Fortney et al, 1994) which aimed to recruit equal numbers of current COC users, former users and never users in women aged 40-54, only found 7 current users in a total sample of 352 which included predominantly former users. Whilst some cross-sectional studies have included older women users in their wider age range, the low numbers of perimenopausal users has resulted in few cross-sectional or longitudinal studies being able to look at this particular user group alone. Three cross-sectional studies including perimenopausal women currently using COCs have not found differences compared to non-users (Masaryk et al, 1998; Tuppurainen et al, 1994; Johnell and Nilsson, 1984).

A limited number of randomized controlled trials have been able to investigate women in the perimenopausal period (Gambacciani et al, 2000; Gambacciani et al, 1999; Volpe et al, 1997); Gambacciani et al, 1994a and Gambacciani et al, 1994b; Shargil, 1985. The Gambacciani study group randomized oligomenorrheic women in all four studies to a COC (20mcg EE/150 mg desogestrel or 30mcg EE/75 mg gestodene) or calcium (500mg/day). In two of the studies normal age matched menstruating women were included as controls who received no treatment. After a two-year follow-up oligomenorrheic women in the COC group had increased BMD in the neck, wards triangle and trochanter, with no differences in BMD found compared to the normal menstruating control group (Gambacciani et al, 1994b; Gambacciani et al, 2000). The

studies not including normal menstruating controls also found increases in the COC treated groups compared to the oligomenorrheic women treated with calcium alone. Two further studies (Shargil, 1985; Volpe et al, 1997) also found that BMD was maintained (Shargil, 1985) or increased (Volpe et al, 1997) in women treated with COCs.

Postmenopausal women

Cross-sectional studies in postmenopausal women have found that there is limited evidence to suggest that history of COC use confers any benefit in BMD post-menopause (Sultana et al, 2002; Forsmo et al, 2001; Grainge et al 2001; Pasco et al, 2000; Masaryk et al, 1998; Ulrich et al, 1996; Fortney et al, 1994; Berning et al 1993; Kris-Silverstein et al, 1993; Melton et al, 1993; Murphy et al, 1993; Hansen, 1991; Stevenson et al, 1989; Lindsay et al, 1986). In a recent systematic review of BMD and COC use (Martins et al, 2006) found that in cross-sectional studies of postmenopausal ever users, most studies included in the review were non-significant, six found higher BMD in ever users of COCs and one found lower BMD values (Hansen, 1991).

One randomized controlled trial in postmenopausal women (Taechakraichana et al, 2000) randomized women to receive either COCs (30mcg EE/150 mg desogestrel) or Hormone replacement therapy (HRT). After a one-year follow-up both groups showed significant gains in all sites measured. A second prospective study followed up women for 12 years from a baseline where they had reached natural menopause within the last 3

years. Although BMD was higher in ever-users at baseline, by the 12 year follow-up no difference was found between users and never users.

In summary, existing evidence suggests there are benefits in use of COCs with users gaining or maintaining their BMD in perimenopausal and menopausal women.

Overview summary of COC use and fracture risk/BMD outcome

The association between COC use and fracture is inconsistent. More data will become available as past users of COCs move into menopause. Although COCs have been available since the 1960s the change in formulation has meant that more evidence is required to establish if there is a link between low-dose COC formulations and fracture risk.

COC use and BMD outcome appears to vary by age-group with few differences found in adult current users in both prospective and cross-sectional studies compared to control non-users. However, some very low-dose COCs may cause small decreases in BMD. In perimenopausal and menopausal women existing evidence suggests there are benefits in use of COCs with users gaining or maintaining their BMD. There is growing concern, as the body of evidence related to adolescent users of COCs use increases, that low-dose COC formulations may be detrimental to BMD in an age group who are yet to reach peak bone mass.

1.6.3 Depot medroxyprogesterone acetate

1.6.3.1 Background and historical perspective

Depot medroxyprogesterone acetate (DMPA) is a long-acting, synthetic, progestin-only injectable contraceptive. Marketed as Depo-Provera[®], it is available in over 90 countries and is a widely used method of contraception in Africa, Thailand and New Zealand and has been widely available in developing countries including South Africa since 1964. Although first reviewed by the US FDA in 1973, only 20 years later in 1992 was final approval granted, after concerns regarding risk of development of cancer were addressed (Kaunitz, 1994). Since receiving FDA approval, DMPA use has increased in the developed world. The number of woman using the method in the United Kingdom (UK) rose from 40,000 in 1993 to 270,000 in 1996 (Bigrigg et al, 1999), however the percentage using the method in the UK has stayed constant at 3% of contraceptive use between 2000 and 2007/08 (Lader and Hopkins, 2008). Hormonal contraceptive injections are increasing in popularity among younger women in particular adolescents (Margulies and Miller, 2001; Chotnopparatpattara and Taneepanichskul, 2000; Davis, 1996; American Health consultants, 1994). In South Africa injectable hormonal contraception continues to lead over other methods, with 50% of women using a method choosing an injectable hormonal method. Of these injectable users, just over half (58%) are using Depo Provera[®] and slightly less (42%) using Nur-isterate[®] (Department of Health, South Africa, 2006). In addition, the South African Demographic and Health Survey (SADHS) found that a higher proportion of younger women were using the injectable method whilst methods such as sterilisation and COCs are being used less frequently (Department of Health, South Africa, 2003, 2006).

DMPA is administered as an aqueous microcrystalline suspension as a dose of 150 mg as a deep intramuscular injection at 3-monthly intervals. Its primary mechanism of action is by causing anovulation via inhibition of pituitary gonadotrophin. This occurs through the disruption of the hypothalamic-pituitary-ovarian axis. In addition there is modification of the endometrial lining and cervical mucus (Mishel, 1996). At the hypothalamic level, gonadotropin-releasing hormone frequency increases, whereas at the pituitary level, the lutenising hormone (LH) surge is absent, and Follicle Stimulating hormone (FSH) is inhibited. Ovarian oestrogen production is suppressed leading to the reduction in synthesis and secretion of ovarian estradiol (Mishell, 1996) and long term users commonly have plasma estradiol levels that are at or below normal levels present in the early follicular phase (Ortiz et al, 1977). Other studies have found that the estradiol levels can be similar to those of post-menopausal women which could be lower than 100pmol/L, particularly if the woman is amenorrhoeic (Gbolade et al, 1998). Although menstrual side effects vary between women, many women become amenorrhoeic after prolonged use (1 year or more) and have hypoestrogenism (Ortiz et al, 1977). Amenorrhea can occur as early as the first three months after the first injection and in one study found 8% of women becoming amenorrhoeic after the first injection and 45% by one year of use (Belsey, 1988). As estradiol is important in bone maintenance the amenorrhoeic woman with low estradiol levels may be at greater risk of bone loss. The loss of BMD in the case of DMPA has been associated with estrogen deficiency and concerns were raised that long-term users of DMPA could be at risk for postmenopausal osteoporosis and that women using DMPA should discontinue the method before reaching menopause (Scholes et al, 2005).

Many studies have investigated the role of DMPA and its effect on BMD with the concern that the suppression of estradiol may lead to osteopenia and ultimately cause an increase in risk of fractures in older women. The studies investigating the effect of DMPA on bone mass first appeared in the literature over a decade after the first reports on the possible relationship between COCs and BMD. The first documented study addressing DMPA and BMD was published in 1991 (Cundy et al, 1991). This cross-sectional study was conducted among 30 adult current users of DMPA in New Zealand and compared pre-menopausal users with a median use of at least five years with non-user controls in the same age range. Results showed that users of DMPA had a significantly lower femoral (6.6%) and vertebral (7.5%) bone density. As the first study published and a British Medical Journal (BMJ) publication it received a great deal of attention and raised concerns regarding use of the method. The difference in bone density values was too small to pose a fracture risk and BMD values were still higher than post menopausal women. After publication, a letter raising concerns about the methodology used by the authors was published in the BMJ. The study was criticized for poor matching of cases and controls and self selection bias (Szarewski et al, 1991). In addition it was commented that there was no baseline data for the women, the sample size was small and smoking prevalence was much higher in the DMPA group. Since 1991 numerous further studies have added to the body of information and evidence in this area and these will be discussed in this section of the literature review.

1.6.3.2 DMPA and fracture risk

Although there is a large body of literature now available with BMD as an outcome in DMPA users, there has only been one report on fracture risk in users of DMPA (Lappe et al, 1997). This study measured BMD in the heel bone using quantitative ultrasound (speed of sound). DMPA use at baseline was associated with an increased risk of stress fracture following an 8-week follow-up. However once adjustment for speed of sound had been made, at baseline, the association with DMPA became non significant. This study was given a quality rating of “II-2 poor” in a recent review (Curtis et al, 2006) due to the limitations of the data.

1.6.3.3 DMPA and BMD outcome

Use of DMPA and BMD outcome in adult premenopausal women

Cross-sectional studies

Many studies published on predominantly adult current users of DMPA have been cross-sectional (Rosenburg et al, 2007; Albertazzi et al, 2006; Shaarawy et al, 2006; Tharnprisarn and Taneepanichskul, 2002; Wanichsetakul et al, 2002; Ott et al, 2001; Perrotti et al, 2001; Petitti et al, 2000; Bahmondes et al, 1999; Tang et al, 1999; Scholes, 1999; Cundy et al, 1998; Gbolade et al, 1998; Pavia et al, 1998; Taneepanichskul et al, 1997a; Taneepanichskul et al, 1997b; Virutamasen et al, 1994; Cundy et al, 1991).

Most cross-sectional studies have shown negative effects of DMPA on BMD (Rosenberg et al, 2007, Albertazzi et al, 2006; Shaarawy et al 2006; Wanichsetakul et al, 2002; Pettiti et al, 2000; Scholes et al 1999; Tang et al, 1999; Cundy et al 1998; Pavia et al 1998; Cundy et al, 1994). Fewer have shown no effect of DMPA on BMD (Perrotti et al, 2001;

Ott et al, 2001; Bahmondes et al, 1999; Gbolade et al, 1998; Taneepanichskul et al, 1997a & 1997b; Virtumasen et al, 1994). No study has reported or shown a positive effect of DMPA on BMD. A number of cross sectional studies have been able to analyse the data further to look at the effect of DMPA on BMD by duration of use of DMPA (Albertazzi et al, 2006; Shaarawy et al, 2006; Tang et al, 1999; Scholes et al, 1999; Cundy et al, 1998).

Following the first publication investigating DMPA and BMD in 1991 (Cundy et al, 1991), Cundy provided further evidence of a negative effect of DMPA on BMD (Cundy et al, 1998). In this study the BMD of 200 current users of DMPA was measured at the lumbar spine and compared with 350 non-user controls. This study included the original 30 DMPA users from the 1991 study. Bone density was significantly lower in DMPA users. Within the users sample women who had commenced DMPA over 20 years of age and had used it for less than 15 years had significantly higher BMD values compared to the users who had used between 16-26 years of use and started at < 20 years. This study reported that length of use was associated with lower bone density, however the relationship was weak. This study was again criticized in a letter (Szarewski and Mansour, 1999) for similar reasons that the 1991 study drew criticism. However, further studies followed in agreement with Cundy's findings adding to the body of evidence that current use of DMPA was detrimental to BMD.

Scholes et al, (1999) recruited 457 women in a population based study of whom 183 women were using injections and 274 were not. BMD was measured at femoral neck,

trochanter, spine and whole body. This study observed a relationship between length of use and decrease in BMD with young women (aged 18-21) using DMPA for over two years being most affected. However, there were no significant differences in mean bone density at any site measured in women over 21 years of age.

Wanichsetakul et al, 2002 measured BMD at the lumbar spine, femurs and forearms in women who had used DMPA for at least 2 years. DMPA users were matched by age, body mass index (BMI) and lifestyles with non-users of any steroid hormonal contraceptives for at least 6 months and found that the lumbar spine was significantly lower in DMPA users compared to the controls, however, there was no differences in BMD noted at the 3 sites measured in the femur and forearm. No information was given on the effect of length of use and reduction of BMD. In addition this study also included COC users where, as discussed earlier, there was no difference found compared to the control group at any of the three bone sites measured. Tang et al (1999) found that BMD was reduced in both lumbar spine and femoral neck in 67 DMPA users with at least five years of use compared with never users. Pavia et al (1998) found that the mean bone density in 72 users was significantly lower than the control group in all sites evaluated (hip, spine, trochanter and wards triangle). However, multiple regression showed DMPA was only associated with BMD in the lumbar spine and that BMI, age and length of amenorrhea were associated with lower BMD at the femoral neck.

In response to concerns raised by the many studies published and their disagreement in effect, the WHO funded a multi-centred cross-sectional study conducted in seven centres

in three regions of the developing world between 1994 and 1997 (Pettiti et al, 2000). This study included DMPA, COC and levonorgestrel implant users who had at least 24 months of lifetime use of one of these methods and they were compared to never users of hormonal contraception or less than 6 months of life time use. The age range of women recruited was narrow with only women within the five-year age range of 30-34 included. The study included 2474 women in total and found that forearm BMD was significantly lower in short-term, current users of DMPA, compared to never users. The changes observed were found to be less than one standard deviation (SD) from the mean of never users. Although short term current users were found to have a lower BMD compared to never users of hormonal methods, this difference was not found in the long term users of DMPA, and the authors commented that this could indicate that these initial decreases were reversible and there could potentially be a return to never user levels after long periods of continued use. The median length of use was 3-5 years in this study and the authors suggest that only a longitudinal study following users for a long duration could really adequately answer the question of recovery during use.

More recent studies (Rosenberg et al, 2007; Albertazzi et al, 2006; Shaarawy et al, 2006) have also found DMPA to lower BMD, however Shaaraway (2006) found that lumbar spine BMD was not significantly different in short term users of DMPA (<1year) compared to non-users. In this study only users of between 1 and 2 years and > 5 years recorded lowered BMD (9% and 11.8% respectively). Albertazzi et al, (2006) measured hip and spine in 218 DMPA users who had also used other methods of contraception. The prevalence of a T score below -2.5% was 5%. Woman who had used DMPA before

COCs were found to be most compromised in BMD. The authors suggested that this information needs to inform the debate on switching women from DMPA to COCs due to concerns about bone density. In one of the largest and most recent studies conducted in South Africa (Rosenberg et al, 2007), 3487 women had ultrasound measurements taken of the left calcaneus. Current users of injectable contraception (DMPA and NET-EN) had the lowest ultrasound measurements.

Cross-sectional studies finding no significant effect of DMPA on BMD have predominately measured forearm BMD (Ott et al, 2001; Perrotti et al, 2001; Bahmondes et al, 1999; Taneepanichskul et al, 1997a; Virtumasen et al, 1994). Perrotti et al (2001), in a cross sectional study of women who had used either DMPA or COCs for at least two years compared to a control group of never users found no difference in forearm BMD. Taneepanichskul et al (1997a), found no difference in BMD in long term users of DMPA and Norplant. The authors concluded that any initial loss of BMD associated with use of DMPA had been recovered during long-term use. In a similar study by the same researchers (Taneepanichskul et al, 1997b) there was again no difference between DMPA users who had used the method for at least 3 years compared to IUD user controls. Tharnprisarn et al (2002) found no statistically significant differences in forearm BMD between Thai women using either DMPA or COCs for a mean of approximately 2 years. Bahmondes et al, (1999) measured BMD in two different sites in the forearm in 50 DMPA users of approximately one year and 50 never users of hormonal contraception. A lower but non-significant decrease was found in the midshaft of the forearm of the DMPA users and this became a significant difference in the distal forearm. Pettitti et al

(2000) as mentioned earlier found a significant difference between DMPA users and controls only in the forearm of short term current users, there was no difference in long term users.

Fewer cross-sectional studies measuring BMD at central sites (lumbar spine, femoral neck and hip) have found no effect of DMPA on BMD (Walsh et al, 2008a; Gbolade et al, 1998; Virutamasen et al, 1994). Gbolade et al (1998) measured spine and femoral neck in women who had used DMPA for between 1 and 16 years with a median of 5 years. These women were compared to a database of values of over 3000 women and BMD in these sites was only minimally below the population mean for the lumbar spine and there was no significant difference in the femoral neck between the two groups. This study also looked at length of DMPA use and found a very weak negative correlation without statistical significance. There was also no clear relationship found between serum estradiol level and length of DMPA use. The authors suggested that any bone loss would occur in approximately the first three years of use and a steady state would be reached thereafter. They concluded that there was no clinically adverse effect of DMPA on BMD. Virutamasen et al (1994) conducted a cross-sectional study that included 75 long term DMPA users (>3 years of use). No difference was found in BMD at the femoral neck between DMPA users and non-user controls even in the group that consisted of users of greater than 7 years. One of the few case-control studies (Walsh et al, 2008a) matched 100 DMPA users with control non-users and took a single measurement at the spine and hip and found in the 35-45 age group there was no difference in BMD.

In summary the cross-sectional studies have generally shown decreases in BMD with use of DMPA in central sites such as the spine but not consistently with the hip. In most studies where forearm BMD has been measured, there appears to be minimal or no change in BMD. Decrease in BMD appears to be linked to duration of use with those using DMPA for longer being more compromised. Studies that have suggested that there may be long term recovery during use have measured the forearm where generally no changes have been found in BMD (Petitti et al, 2000).

Prospective studies

There is now a strong body of evidence from longitudinal data that shows that DMPA compromises BMD in adult current users. Some of these studies have enrolled and followed up adult women as new users of DMPA (Berenson et al, 2008; Clark, 2006; Kaunitz et al, 2006; Berenson et al, 2004; Clark et al, 2004; Berenson et al, 2001; Naessen et al, 1995). Some have enrolled continuing DMPA users (Cundy et al, 2003; Merki-Feld et al, 2003; Tang et al, 2000; Cundy et al, 1994) and some have included a combination of new users and continuing users (Scholes et al, 2002). Of these studies a small number have been RCTs (Berenson et al, 2004; Cundy et al, 2003; Berenson et al, 2001; Naessen et al, 1995). In these studies, women have been followed for between 1 and four and a half years. Almost all longitudinal studies found a negative effect of DMPA on BMD. In studies of new initiators of DMPA one RCT (Berenson et al 2001) followed-up 33 new users of DMPA for a year and found a mean loss of BMD of 2.74% over 12 months compared to non-hormonal contraceptive user controls (n=59) who lost

0.37% in the lumbar spine. COC users were also included in this study (n=63) and they demonstrated a gain in BMD compared to controls. The same authors (Berenson et al, 2004) conducted a further RCT and found that the loss of bone was linear over a two-year period with a total loss of 5.7% in the spine after two years. Recently, Berenson et al (2008), conducted a 3-year follow-up study in women aged 16 to 33. DMPA users lost 3.7% BMD in the spine and 5.2% in the femoral neck compared to gains in the non-user controls. The loss was greater in the younger users (16-24 years) who lost 4.2% at the spine, and 6.0% at the femoral neck. In a 2- year follow-up Clark et al, (2004) reported a similar loss of 5.8% in the hip and 5.7% in the spine in 178 DMPA users. Non-hormonal contraceptive users maintained BMD in this study. These women continued their follow-up for 48 months (Clark et al, 2006), when it was found that hip BMD declined further to a loss of 7.7% and spine a loss of 6.4%. The BMD of controls however also declined by 1.6%. This study found a slowing in loss of BMD, and after 48 months of use the BMD loss slowed to 0.6% per year.

Kaunitz et al (2006), conducted a 7 year prospective matched-cohort study. This study aimed to follow-up 248 new users of DMPA for 240 weeks of treatment (approximately 4.5 years) and 360 non-hormonal user controls. Women discontinuing DMPA before the 240 weeks, and those discontinuing at 240 weeks continued to be followed up to look at recovery. For DMPA users continuing in the study until 240 weeks, BMD had decreased from baseline by -5.16% in the hip and a similar loss of -5.38% in the spine. For non-hormonal users the change in spine and hip was negligible. The rate of loss was more pronounced in year 1 for DMPA users.

Only one longitudinal study of new users found stable values for BMD during follow-up (Naessen et al, 1995). This RCT randomized 22 women to use either DMPA or Norplant. BMD was measured at the forearm and 18 women completed the six month follow-up. BMD decreased in the DMPA group by 0.41% but this was not significant. Bone metabolism markers indicated an increased turnover in bone resorption, however this was only significant in serum calcium. This study was limited by its short duration of follow-up and the small number of women involved.

The second group of prospective studies have followed-up existing users of DMPA (Walsh et al. 2008b; Merki-Feld et al, 2003; Cundy et al, 2003; Tang et al, 2000; Cundy et al, 1994). The first longitudinal study published (Cundy et al, 1994), included 22 women who were continuers of DMPA. This group was compared with a group of 18 non-user controls. Baseline values showed the lumbar spine was lower by approximately 9% and femoral neck by 5.7% in the user group compared to the controls. At follow-up (median 12 months) there was no change in femoral neck or spine in either group.

Merki-Feld et al, 2003 recruited 35 existing users of DMPA who had used the method for a mean of 42 months and 10 non-exposed controls. Twenty three DMPA users completed two years follow-up and in this group there had been an increase in trabecular bone mass of 0.6% but a decrease of 0.1% in cortical bone mass. This decrease however was not different from that seen in the control group. After one year of follow-up women with a trabecular bone loss of more than 1% received treatment with calcium or estrogen during

the second year of follow-up. Over the two-year period there was no significant change found in the BMD in both sites in the untreated DMPA group. There was no comparison with the controls at year 2 as 6 of the 10 controls had dropped out of the study.

Thirty-eight long term users of DMPA with low spinal BMD were randomized to receive conjugated estrogen (0.625mg daily) or placebo (Cundy et al, 2003). At the end of two years the treatment group gained 1% spinal BMD whilst the placebo group had lost 2.6% and this difference was significant. One study that had originally reported on a cross-sectional population (Tang et al, 1999) followed up 64 of the original 67 women in the study for 3 years (Tang et al, 2000). These women were already long term DMPA users (of approximately 5 years) on recruitment into the cross-sectional study. In the prospective component of the study women lost substantially less BMD than had been anticipated from the values found in the cross-sectional study. The rate of bone loss per year was not related to the length of DMPA use. BMD decreased over the 3 year period in the spine, hip and wards triangle and was significantly lower compared with 218 never users of hormonal contraception. BMD in the trochanter showed a non-significant gain in DMPA users. The conclusion put forward by the authors was that the initial reduction of BMD would level off after 5 years. The rate of loss per year was found to be far lower than other studies for the spine (0.442%) and hip (0.403%), compared to other longitudinal studies that have shown BMD losses of between 2-5%. This study therefore did not suggest recovery during use, but rather stabilization of loss of bone. They postulated that BMD would remain lower than in a population of comparable non-users however would not continue to decline on continued use.

Scholes et al (2002) followed- up 183 women for three years and included women who were existing DMPA users, but also managed to include 43 women who were new users of DMPA in that they were enrolled after their first injection and prior to their second and so could be described as initiators. BMD at the spine and hip decreased every six months in the users compared to the 258 non-users. The annualized mean rate of change in DMPA users was -0.87% at the spine and -1.12% at the hip compared to +0.40% in the spine and -0.05% in the hip of the controls.

Only one study of continuing users has found no difference between DMPA users and controls after 3 years follow-up (Walsh et al, 2008b). However due to method discontinuation only 23 of the original 50 users attended the 3 year follow-up and an additional 14 attended but had discontinued. There were also no interim measurements between baseline and 3 years.

In summary most longitudinal studies have shown that current adult users of DMPA will lose BMD. This loss appears to commence from about 6 months of use and appears linear for the first 2 years. Longer follow-up in several studies indicates that the rate of loss plateaus and by five years the loss may stabilise although recovery does not occur during use.

Use of DMPA and BMD outcome in adolescents and young women

Some studies have investigated the effect of DMPA on BMD primarily in adolescents and young women (Cromer et al, 2008; Cromer et al, 2005; Scholes, 2005; Cromer et al, 2004; Rome et al, 2004; Scholes et al, 2004; Lara-Torre et al, 2004; Busen, 2003; Cromer et al 1996). Other studies have analysed the younger woman as a sub-set of a sample in a wider age range (Berenson et al, 2008; Walsh et al, 2008; Tharnprisan et al, 2002; Scholes et al, 1999; Cundy et al, 1998). Most of the studies including adolescents have found a negative association between DMPA and BMD (Cromer et al, 2005; Scholes et al, 2005; Cromer et al, 2004; Lara-Torre et al, 2004; Busen, 2003; Scholes et al, 1999; Cromer et al, 1996).

Cross-sectional studies

There exist four published cross-sectional and one case-control study of adolescent users of DMPA (Walsh et al, 2008a; Scholes et al, 2004; Tharnprisan et al, 2002; Scholes et al, 1999; Cundy et al, 1998). Scholes et al (2004) found a non significant reduction in the BMD of the hip and spine in 81 adolescent DMPA users (14-18 years) compared to controls. Women in this study had used between 1-13 injections with a median of 3. The Tharnprisan et al, (2002) study refers to adolescent and young Thai girls in the title of the publication, however, this is slightly misleading as although all 60 women in the sample were 30 years or younger, only 3 DMPA and 3 COC users were under 20 years (adolescents), with almost half over 25 years. This study found no difference in the forearm BMD of DMPA and COC users all of whom had used the method for at least two years. A sub analysis of young women in Cundy et al (1998), found that the lumbar spine Z score was lowest in the 26 women who commenced DMPA use at 20 years or

younger. Scholes et al (1999), found in age specific comparisons that the youngest group (18-21 years) were the most affected with a significant decrease compared to controls at all anatomic sites (neck hip, spine, whole body). Above 21 years there was no difference between DMPA users and controls and the age adjusted mean BMD was significantly higher in the unexposed groups in the spine and trochanter only. Spine and hip BMD was measured in a subset of 50 women aged 18-25 who started DMPA prior to 20 years in a case-control study (Walsh et al, 2008a). DMPA use was associated with a 5% lower BMD compared to controls.

Prospective studies

The earliest longitudinal study including adolescent users of DMPA, Norplant, COCs and non-user controls was published in 1996 (Cromer et al, 1996). In a limited 2 year follow-up in this study, DMPA users had significantly lower BMD compared to the Norplant and non-user control groups. The sample sizes in the different groups were very small (DMPA n=15) and by the second year of follow up only 15 women of the original 48 recruited across all groups were still participating in the study. Further longitudinal work by the same author (Cromer et al, 2004) looked at a much larger sample of 370 adolescents aged 12-18 who self selected for either a low dose (20 mcg EE) COC, DMPA or controls who received no hormonal method. After a one-year follow-up the DMPA group showed mean percent change of -1.4% in the spine compared to controls. Over a 12 month period there was evidence of increased bone formation and resorption in the control group when compared to that in the DMPA and COC groups. There was no relationship between the biochemical markers and BMD at the lumbar spine or the

femoral neck. A two-year follow-up by the same researchers (Cromer et al, 2008) included postmenarcheal girls aged 12-18 using DMPA (n=58), COCs (n= 187) and non-user controls (n=188). Measurements were taken at the spine and femoral neck and after 24 months the mean % change in the DMPA group was -1.5% in the spine and -5.2% in the femoral neck compared to increases in the COC and non-user group. It was reported that most BMD loss in DMPA users occurred in the spine in year 1 with only a change of -0.1% recorded in year 2.

Cromer et al (2005) looked at estrogen supplementation in a group of adolescents commencing DMPA. Initiators of DMPA were randomly assigned to receive monthly injections of estradiol cypionate or placebo. Participants were followed up at 1 and 2 years. At two years, those receiving estrogen supplementation increased BMD in the spine +2.8% and femoral neck +4.7%, compared to a loss of 1.8% in the spine and 5.1% in the femoral neck of those DMPA users without supplementation. As seen in other studies including adolescents, less than one-third completed the two-year follow-up.

Lara-Torre et al, (2004) also found a statistically different decrease in BMD of the lumbar spine between new users of DMPA and controls after six months (-3.02%). After two years this decrease increased to -6.81% relative to controls. Comparison with the COC users found that although the BMD of DMPA users was lower compared to COC users these differences only reached significance at 12 and 18 months. Although 148 adolescents aged between 12 and 21 years commenced the study, only 32 completed

follow-up. This study recognized the low rate of compliance with contraception making studies of contraceptive users in this age group difficult to complete.

Scholes et al, (2005) continued to follow women who entered the cross-sectional study in 1999 (Scholes et al, 1999) and these women continued in the study for 36 months. The DMPA users showed significant declines in BMD at the hip and spine but not the whole body, relative to non users. In this study new DMPA users lost bone more rapidly compared to prevalent users. Berensen et al, (2008), analysed a subset of adolescents and young women age 16-24 years in their 3-year follow-up of new DMPA and COC users. This younger group of DMPA users lost more BMD when compared to the women aged 25-33 years.

Busen (2003) recruited 22 girls aged 15-19 of whom only 6 continued participation at one year and four at year 2. At one year all six DMPA users had shown a mean decrease in BMD of 3.31% in the femoral neck and 3.52% in the lumbar spine. No control groups were included and 2 of the four girls had shown recovery of BMD with one returning to baseline and the other showing a slight increase in BMD at 2 years. This study reported that it was compromised by small sample size and attrition of the sample.

In summary there is similar evidence of decreases in BMD in young women current users of DMPA as in adult users. Decreases as well as lack of increase in BMD are important in adolescents where increases in BMD should be found as age group is still progressing to peak BMD. Many of these studies have been affected by poor follow-up due to young women discontinuing use of their contraceptive methods.

Use of DMPA and BMD outcome in older women (perimenopausal and post menopausal)

The populations investigated in studies looking at the effect of DMPA have included women using the method in their 40s but there is limited information in this age group specifically. The age ranges of women in some studies have included users up to the age of 52, however the numbers have been small. In one cross-sectional study older users were disaggregated in the data (Gbolade et al 1998) and no differences were found in BMD in women aged between 40-49 and a slightly older group of 50-52 compared to a normal population mean in the lumbar spine and femoral neck. The population of women in the Tang et al (1999) cross-sectional study were generally older than other studies with a mean age of 43 years. In this study those who had used DMPA for 5 years or more had significantly lower BMD in the spine, femoral neck, trochanter and ward's triangle compared to never users. From the initial cross-sectional sample of 67 women, 59 were followed up in the 3-year prospective component of the study (Tang et al, 2000). Small losses were noted of less than 1% in all sites but not the trochanter. In addition there was no effect seen with duration of use aside from a weak negative between BMD in the femoral neck and duration of use. One longitudinal study (Sanches, 2008) followed up women who had been long-term users of either DMPA or the IUD until menopause. This study found no difference in forearm BMD between these two groups at each of the three one-year follow-ups post-menopause.

In summary there is limited data available on DMPA use in this group of older women due to the low prevalence of use in this age-group. Information in this age group has come from studies including a wider age range of women. However, loss of BMD has been found in these older DMPA users.

Recovery of BMD Post discontinuation of DMPA

The previous sections have addressed current users of DMPA and how their BMD has been affected. Several studies have tried to investigate potential long term consequences of DMPA use. Some studies have looked at ex-users of DMPA to see if BMD has returned to normal population values and therefore inferred that recovery may have taken place (Rosenberg et al, 2008; Pettiti et al, 2000; Orr-Walker et al, 1998). Women have also been followed-up from the point of discontinuation of DMPA to look at potential recovery and additionally, the rate of recovery in terms of the timeframe taken for a full recovery to baseline values (Walsh et al, 2008b; Clark et al, 2006; Kaunitz et al, 2006; Scholes et al, 2005; Scholes et al, 2002).

Cross –sectional studies including ex-users of DMPA

A number of studies have looked at the history of DMPA use in past users of the method. These studies have generally found few differences between ex-users and never users of DMPA (Rosenberg et al, 2008; Pettiti et al, 2000; Orr-Walker et al, 1998). Pettiti et al, (2000) found no difference in forearm BMD between past users and never user controls, even among those who had used the method for 4 or more years. The largest cross-sectional study (Rosenberg et al, 2007) included 3487 women of whom 3151 had ever

used injectable contraception (DMPA and NET-EN). There was no difference in the BMD of the calcaneus in never users and ex-users of injectable contraception of at least 2-3 years.

By investigating post menopausal women who continued use of DMPA up until natural menopause, we could see if DMPA use had long term effects, however, only one study has investigated this (Orr-walker et al, 1998). This study included 34 post menopausal women who had used DMPA in the past. Most of these women had commenced DMPA in their later reproductive years (median age 41 years) and women had used DMPA for a median of 3 years. The median age of discontinuation was 45 years (probably before menopause). At the three sites measured that there was no significant difference in BMD, however, they did report a trend towards lowered BMD in these former users (1.6% in the lumbar spine, and 3.1% the femoral neck). There was no association between duration of use of DMPA, the age at discontinuation and the point of discontinuation relative to the menopause. This study concluded that the use of DMPA did not have a residual effect on BMD and therefore should not pose a risk to women in their post- menopausal years.

Longitudinal Studies of women post discontinuation of DMPA

Cundy et al, (2002) looked at 16 women aged 45-53 years who had used DMPA continuously for a minimum of five years and who had reached menopause. This group was compared to 15 controls with no history of DMPA use. These women were followed over 3 years to assess early postmenopausal bone loss. At baseline the women using

DMPA were found to have a mean BMD of 7% lower at spine and 9% lower at the hip compared to controls. The early menopausal bone loss was found to be rapid in the non-hormonal contraceptive user control group, with 6% loss of BMD from both sites over the 3 year period. The women who had used DMPA up to menopause showed a negligible change in BMD over the same period of time. The percentage change was statistically significant at both the spine and hip between the two groups. Given the changes observed over the three year period, Cundy, (2002) estimated that the deficit in BMD in DMPA users, relative to control subjects, would have been eliminated completely at the lumbar spine and by more than one-half at the hip. Further into menopause it was assumed that these differences would be negligible. In conclusion the authors stated that women who would normally choose to use this method of contraception until menopause should not be discouraged from doing so. The women in this study commenced DMPA use after 25 years of age and therefore caution was expressed about applying this conclusion to women who commence the method during their teenage years, where there is less evidence for issues of recovery in the long term.

Longitudinal studies in premenopausal women during use and post-discontinuation of DMPA

Some studies have measured BMD in premenopausal (including adolescent) women during use and post discontinuation (Walsh et al, 2008b; Clark et al, 2006; Kaunitz et al, 2006; Scholes et al, 2005; Scholes et al, 2002. Walsh et al (2008b) measured hip, spine and femoral neck in 14 discontinuers of DMPA and found no difference between this group and controls at the 3-year follow-up visit. Clark et al, (2006) followed up 178

DMPA users post discontinuation and found that BMD increased between 0.3% to 2.0% per year depending on the length of DMPA use and bone site. Eighteen months after discontinuation the women with the longest recorded DMPA use still remained 4.7% and 2.9% lower at hip and spine compared to baseline.

Cundy et al, (1994) measured BMD in a small group (n=14) of adult women who had been users and had discontinued DMPA and found that despite weight loss which usually decreases BMD, the BMD increased by 3.4% in the spine after a one-year follow-up after cessation of use. A sub-group of 8 discontinuers were followed for a further year and the increase continued at the same rate, resulting in a total increase of 6.4% over two years. It was suggested that BMD loss was probably completely reversible within 2 years of discontinuation. A prospective study followed up DMPA users for 3 years measuring BMD every 6 months (Scholes et al, 2002). Discontinuers of the method continued to be followed-up and in total 110 women discontinued the method during the follow-up and showed a marked increase in BMD for each six-monthly interval of $+0.0067 \text{ gm/cm}^2$. After 30 months the mean bone density of the DMPA discontinuers was similar to that of non-users.

Kaunitz et al, (2006) was able to follow-up 44 adult women for 96 weeks of post-discontinuation and at this point change from baseline was -0.20% for total hip and -1.19% for lumbar spine. Duration of exposure to DMPA appeared to affect speed of recovery, in that women with longer periods of use took longer to recover, compared to women who used DMPA for shorter lengths of time.

Scholes et al, (2005) undertook the first study that has been able to produce data indicating recovery of BMD in young women using DMPA and were able to follow-up adolescents post-discontinuation. Women who continued in the study but discontinued DMPA exhibited significant increases in BMD relative to non-users at all anatomic sites. In this study over 80% of the adolescents had discontinued DMPA use during the 36 month follow-up period and this group consisted of 62 of the original 80 baseline users. The authors concluded that the gains in BMD post discontinuation provided evidence that the loss of BMD could be reversed.

In a systematic review of studies published between 1996 and 2006, Kaunitz et al (2008), evaluated changes in BMD after discontinuation of DMPA. They reported that BMD values started to recover from as early as 24 weeks post discontinuation and continued during the reported follow-up period which varied depending on the study.

Recovery of BMD in long term users of DMPA

Most studies have found that long term users remain compromised with respect to their BMD. In only one study (Pettiti et al, 2000) was there a suggestion that long term users could recover BMD. This may have been suggested as this study found that short-term users of DMPA had lower BMD compared to non-users, but this was not found in long term users. However, considering the measurement site used was the forearm, where few studies have found a difference between user and non-users of DMPA, this is not regarded as a plausible theory.

Overview and summary of DMPA use and fracture risk/BMD outcome

Most studies have indicated a negative effect of DMPA on bone mass and those showing minimal or no effect have mainly measured the forearm (Curtis and Martins, 2006). Loss of BMD appears to be greatest in the lumbar spine and femoral neck. The differences have usually been within 1SD of the mean BMD in non-users. Loss of BMD appears to be greater in the first year of use (2-5%) and loss begins to stabilize by five years, with losses in continuing users stabilizing at around 1% a year. There is no longitudinal follow-up of users beyond five years that would provide evidence about whether this loss plateaus in longer-term use. Cross-sectional studies that have included women who have used DMPA for more than five years have lower BMD, but if BMD loss was linear from year one the BMD in these long term users would be expected to be considerably lower. There is only one study (Pettiti et al, 2000) that found no difference in long term users compared to non users in a cross-sectional study. Studies that have followed users beyond discontinuation show that these negative changes are reversible and there is a return to pre-use levels on discontinuation of the method (Kaunitz, et al, 2008). No studies have found a positive effect of DMPA on BMD. In longitudinal studies that have enrolled new users of DMPA, few have been able to follow-up users for more than four years and therefore longer term evaluations will be needed to inform the effect of DMPA on BMD in longer term users.

Limitations of the longitudinal studies include sample size, length of follow-up, discontinuation rates and adjustment for confounding. Some studies have included less

than 20 DMPA users at baseline (Busen, 2003; Cromer et al, 1996; Naessen et al, 1995; Cundy et al, 1994). Discontinuation before the end of the study follow-up period is a general problem in many of the longitudinal studies and in particular those that have looked at adolescents and young women, this further reduces the numbers available for long term follow-up. Discontinuation rates of up to 60% are common in adolescents in these studies (Busen, 2003; Scholes et al, 2002).

Studies that have measured bone metabolism markers concurrently with BMD measurements have found an increased turnover in bone resorption in DMPA users. The quality of studies have been variable and a systematic review of progestogen-only contraception and bone mineral density published in 2006 by Curtis et al, classified few studies as “good” based on the criteria for grading the internal validity of a study using the system for grading evidence developed by the United States Prevention Task Force (USPTF) (Harris et al, 2001).

1.6.4 Norethisterone enanthate

Norethisterone enanthate (NET-EN), marketed as Noristerat[®] is the only currently available 2-monthly injection and is administered every 8 weeks as a dose of 200mg. Its mode of action is more complex than that of DMPA. Primarily there is inhibition of ovulation, thickening of the cervical mucus, prevention of implantation and possibly alteration of tubal function (Fraser and Weisberg, 1981; El-Mahgoub and Karim, 1972). After 60 days, ovulation may no longer be inhibited and the main action is reported to be on the cervix (Fraser et al, 1981). There is limited information available on the effect of

NET-EN on BMD. Only one large cross-sectional study of 3487 premenopausal South African women found current NET-EN users had lower BMD in the calcaneus compared to control never users (Rosenburg et al, 2007). Some published studies on norethisterone (NET) have shown a positive effect on BMD (Horowitz et al, 1993; Eldred et al, 1992; Riis et al, 1990). It is suggested that NET may protect against calcium loss via a direct topic effect on bone or by the action of estrogenic metabolites (Christiansen et al, 1985). NET-EN can be considered to be similar in action to NET as the oestrogen (EN) is added primarily to give it its depot characteristics. Published studies have followed up women on treatment with NET for a year or less and therefore there are no data demonstrating that the positive effect of NET would be sustained in long term use. The lack of data on the effect of NET-EN on BMD may be because the method is not as widely used as DMPA. However, in South Africa this method has increased in popularity in recent years and is often the method of choice for younger women (Dept of Health, 2006). This two monthly injectable contraceptive was introduced later than DMPA in South Africa. In recent years the use of NET-EN has increased considerably in a number of countries especially in South Africa.

1.6.5 Other hormonal methods of contraception

1.6.5.1 Contraceptive implants

A small number of studies have looked at the effect of levonorgestrel releasing subdermal contraceptive implants (Monteiro-Dantas et al, 2007; Bahmondes et al, 2006a; Vanderjagt et al, 2005; Beerthuis et al, 2000; Pettiti et al, 2000; Di et al, 1999; Diaz et al, 1999; Intaraprasert et al, 1997; Taneepanichskul et al, 1997a; Cromer et al, 1996;

Naessen et al, 1995). The products used in these studies included Norplant[®], Jadelle[®] and Implanon[®]. Norplant is a six-capsule subdermal implant releasing 50-85 ug of levonorgestrel per day in the first 9 months of use and about 35ug per day after one year. Implanon[®] is a single-rod etonogestrel-releasing implant and Jadelle[®] is a two silicone rod levonorgestrel-releasing implant. In this section studies will be discussed together as there are fewer reported studies and the age group using implants has mainly included adult premenopausal women. Only one study (Cromer et al, 1996) focused on adolescent users.

Some of these studies included other hormonal methods as comparison groups (Pettitti et al, 2000; Di et al, 1999; Taneepanichskul et al, 1997a; Cromer et al, 1996; Naessen et al 1995). One study conducted in China (Di et al, 1999) used Norplant with a comparison group using a similar levonorgestrel implant but manufactured domestically. The largest of the cross sectional studies (Pettitti et al, 2000), included DMPA and COCs with a non-user control group. The analysis restricted to exclusive users of Norplant (n=308), found the adjusted BMD at the midshaft of the Ulna was significantly lower for Norplant users compared with never users of hormonal contraceptives. This is the only cross-sectional study that reports a negative effect of Norplant on BMD. Taneepanichskul et al, (1997a) compared long-term users of DMPA with a mean duration of use of 31 months with Norplant users (mean duration of use 59 months). There was no non-user control group included. Measurement of BMD was taken at the distal and ultra distal forearm and there was no difference found between the two groups. Inaraprasert et al (1997), found no

difference in forearm BMD between 41 Norplant users aged 19-42 with a mean length of use of 31 months compared to 50 current IUD users.

Looking at the prospective studies, Naesson et al, (1995) included 22 women aged between 20 and 45 years who were randomized to either DMPA or Norplant. Only 18 women had a baseline forearm BMD measurement taken and only 19 completed the 6-month follow-up period. Forearm bone density increased in the Norplant group by 2.94% in the 10 women in the Norplant group compared to a non-significant decrease of 0.41% in the DMPA group. Biochemical indices showed that in DMPA users there were signs of increased bone turnover with increases in bone resorption with increased levels of alkaline phosphatase and osteocalcin. The Norplant group showed an increase in bone formation with increased levels of alkaline phosphatase and osteocalcin. Di, et al, (1999) conducted a prospective study using Norplant and the domestically manufactured implant. In total, 60 women aged between 25 and 40 years were included who were randomised into either group (Norplant n=29 and domestic implant n=31). All women had a BMD measurement taken at one year at the lumbar spine and proximal femur. Both groups showed a significant increase in BMD at one year with a 2.4% increase in the Norplant group and a 2.75% increase in the domestic implant group. In the third prospective study (Cromer et al, 1996), Norplant, DMPA, COC users and control groups, were included, however, numbers were small with a total of 48 women aged 12-21 across all groups. New users of the methods were included and a BMD was taken at one year with an even smaller subsample measured at two years. No COC users were included at 2 years as all had discontinued the method. At one year there was no significant difference

between the user groups in BMD however within groups there was a 1.5% decrease in DMPA users compared to an increase of 1.52% in COC users and a larger increase (2.46%) in the Norplant users. One study compared lactating women who initiated Norplant, progesterone vaginal rings or the IUD (Diaz et al, 1999). There was no difference in spinal BMD between the three groups at 12 months postpartum.

There have been three studies published that have included Implanon (Beerthuizen et al, 2000; Bahmondes et al, 2006a; Monteiro-dantas et al, 2007). Beerthuizen et al (2000), compared Implanon and IUD users and found no difference after two years of use in BMD at a number of skeletal sites. Bahmondes et al, (2006a) included both Implanon and Jadelle and after 18 months of use BMD was found to be significantly lower in the midshaft of the ulna compared with baseline in both implants. However, no difference was found in the distal radius. Similar results were found after 36 months of follow-up in the same study (Monteiro-Dantas et al, 2007). No other skeletal sites were measured.

In summary although most of the studies show no effect of Norplant, these studies were limited by small numbers and different types of users. The largest study (Pettitti et al, 2000) is limited to cross-sectional data, long term users and many different population groups. A Consensus statement and background review published in 1998 (Fraser et al, 1998) was only able to review two published studies at that time (Cromer et al, 1996; Naessen et al, 1995) and reported increase of BMD in users of Norplant. Studies of other implants are inconclusive.

1.6.5.2 Monthly injectables

There are two monthly injectable products currently available (Mesigyna and Cyclofem). Mesigyna is a preparation consisting of 50 ug Norethisterone enanthate and 5ug estradiol valerate. Cyclofem consists of 25mg medroxyprogesterone acetate and 5 mg estradiol cypionate. Two studies have investigated the effect of a monthly hormonal injectable on BMD (Bahmondes et al, 2006b; Von Kesseru et al, 2000,). In a randomized trial (Von Kesseru et al, 2000), 148 women aged between 38 and 50 years were randomized to either Mesigyna (n=49) or IUD (n=99) in a ratio of 1:2. Bone densitometry was only performed on half the women at the end of one and two years of follow-up. Although the study title stated special emphasis on serum lipids and bone density patterns, the primary focus was on serum lipids with minimum information in the results and discussion on the BMD data collected. Results reported there was no difference in BMD at the lumbar spine between the Mesigyna and IUD groups. Although not discussed, follow-up appeared to be poor with only around 50% of either group still using the method at one year and no detail as to how many of those included in the initial subsample for BMD, were still using the method at year 1. The second and most recent study (Bahmondes et al, 2006c) compared women using two kinds of once-a-month injectable contraceptives (Cyclofem and Mesigyna) with a control group of IUD users. This cross-sectional study found no difference in forearm BMD between either of the two injectables and the control group.

1.6.5.3 Progesterone only oral contraceptives

Progesterone only oral contraceptives (POPs) contain a microdose of one of three progestagens (norethisterone, levonorgestrel and ethynodiol diacetate). The main mode of action is by alteration of the cervical mucus and in addition an effect on ovulation. In over half of women (60%) amenorrhoea will result due to cessation of ovulation. The POPs have received little attention thus far with respect to their effect on BMD. This may be due to their low prevalence of use (Guillebaud, 2001). The POPs account for only 8% of the UK oral contraceptive market and in South Africa they are rarely available in the public sector. Only one study has been identified (Caird et al, 1994) which included 9 breastfeeding women using POPs and compared them to 19 women using barrier methods as controls. However half of the control women (n=10) were formula feeding. Although all women showed a significant decrease in lumbar spine BMD at 6 months postpartum, the decrease was significantly lower in the POP users compared to non users. This study did not control for frequency of breastfeeding and with a small number of women, it is not possible to use these data to comment on any relationship between POPs and BMD.

1.6.5.4 Progesterone vaginal ring

Progesterone vaginal rings are inserted into and can remain in the vagina for 90 days. They are ring shaped and around 5-6 cm in diameter. The silastic carrier of the ring releases a controlled dose of progesterone to the vaginal mucosa. Since 1980 research has centered on a levonorgestrel releasing ring of a daily dosage of 20 mg. Only one study has been identified (Diaz et al, 1999) that looks at BMD and use of the progesterone vaginal ring. In this study breastfeeding women (n=28) using the ring were compared to

51 breastfeeding IUD users. No difference was found in BMD at the lumbar spine or femoral neck up to one year post weaning.

1.6.5.5 Intrauterine systems

Only one study (Bahamondes et al, 2006c) has evaluated BMD in users of the levonorgestrel-releasing intrauterine system (LNG-IUS) and compared this group with standard intrauterine device (IUD) users. This cross-sectional study paired long term LNG-IUS users with IUD users by age and BMI. BMD was measured at the ulna and distal radius. No difference was found in BMD values between the two groups.

1.6.6 Review of Study limitations:- Sites measured and equipment used in studies on BMD and hormonal contraception

Although there are numerous studies addressing the issue of hormonal contraception and its effect on BMD, there are limitations with a large number of studies and wide variations between studies in a number of areas including:- sites measured, equipment used; study design; length of follow-up and high rates of loss to follow-up; confounding variables not adjusted for; wide age ranges and lack of or inappropriate control groups. The following section briefly discusses these limitations.

Studies that have investigated hormonal methods of contraception and BMD have measured bone mass density at different skeletal sites. Sites used have included both a range of central and peripheral sites. Central sites have included the lumbar spine, femoral neck and hip whilst peripheral sites include the forearm and ankle. The generally higher costs and greater space requirements needed to measure central BMD sites will no

doubt have been considerations in research studies. Equipment for peripheral measurements such as the forearm, are cheaper to purchase and maintain and are more portable for study requirements. Some studies have used a combination of central and peripheral sites whilst others have used either central or peripheral. There has been some debate in the literature as to whether the peripheral sites are less able to predict fracture risk at spine and proximal femur. Some prospective studies have not demonstrated that the a spinal BMD is superior to peripheral measurement and concluded that forearm measurements predicted future vertebral fractures as well as spinal BMD measurements (Melton et al , 1993; Duppe et al, 1997). However although a large number of studies have confirmed that forearm BMD measurements can predict future fracture risk, many clinicians will continue to prefer to base their advice to patients on spine and hip measurements, since these sites are widely recognized as those where fractures may have the most serious consequences for quality of life and cost of treatment.

Within studies using both central and peripheral sites there have been some differences in the effect of hormonal contraception and BMD. Studies that have used the forearm only have been less likely to find a decrease in BMD in DMPA users (Ott et al, 2001; Perrotti et al, 2001; Bahmondes et al, 1999; Gbolade et al, 1998; Taneepanichskul et al, 1997; Virtumasen et al, 1994). If a decrease in BMD has been found at the forearm in DMPA users then it has not been statistically significant (Naessen et al, 1995). Of the studies using the forearm that have found a negative effect in DMPA users (Pettiti et al, 2000) the change was small and less than one standard deviation. In one study looking at users of hormonal implants (Bahmondes et al, 2006a) although no difference was found in the

radius a significant difference was found at the ulna. Studies that have included both central and peripheral sites have found that changes in BMD have not always occurred equally at all sites (Wanichsetakul et al, 2002).

There are various methods of measuring or estimating BMD and guidelines published in 2007 by the International Society for Clinical Densitometry (ISCD) outline alternative methods to measuring osteopenia (low bone mass) and osteoporosis in pre-menopausal females (Lewiecki, et al, 2007). Although many different sites have been measured, most studies investigating hormonal contraception and BMD have used equipment using Dual-energy x-ray absorptiometry (DXA) as a measurement. Fewer have used single-energy x-ray absorptiometry (SXA) and there are even fewer examples of use of peripheral quantitative computed tomography. Only one recent study has utilized peripheral quantitative computed tomography of the calcaneus (Rosenburg et al, 2007) and this method was chosen as the study sites that had no access to DXA technology

Studies have included both cross-sectional and longitudinal designs but few RCTs. Longitudinal designs have often been varied in type of user and have included both new and continuing users. The length of follow-up has gradually increased as research is showing that the length of hormonal contraceptive use may impact on the rate of BMD loss. The need to follow up beyond discontinuation is now considered key in studies. For adolescents long-term follow up is lacking to investigate any long term effects of hormonal contraception on BMD. Poor follow-up has impacted on sample size, in particular in studies involving adolescents.

As previously described there are many factors affecting bone formation and resorption. It is therefore important to adjust for some of these key factors. Although many studies have adjusted for key variables such as age, BMI and years of lactation, many studies have made no or minimal adjustments. Age ranges have been both wide and narrow and control groups of non-users have not always been included.

The change in COC formulation over the years has required that researchers investigate the new low-dose formulations. These have been found to have a different and opposite effect to the higher-dose formulations available when this area of research became active.

In conclusion this literature review has included and described the history and the current understanding of the effect of hormonal contraception on the BMD of women in adolescence through to the menopause. Although a wide body of work exists and a consensus has been reached on the effect of hormonal contraception such as DMPA, there are still outstanding questions to be answered for COCs and BMD and less researched methods such as NET-EN.

CHAPTER 2.0 MATERIALS AND METHODS

2.1 General outline

This longitudinal study examines the relationship between current hormonal contraceptive use and bone mineral density (BMD) in women aged 15-19 years and 40-49 years. Hormonal and non-hormonal contraceptive users attending the Commercial City family planning clinic, Durban, were asked to take part in the study and once recruited were followed-up for a five year period. Non users of hormonal contraceptives included barrier method and IUD users. At recruitment, clients had a medical history taken and measurements of anthropometry (height and weight). Forearm BMD was measured using dual energy x-ray absorptiometry (DEXA) technique. The admission questionnaire was designed to collect information on socioeconomic variables: education, employment and income. Information was also collected on factors known to be associated with BMD including; pregnancies and lactation history: current and past use of tobacco, alcohol and caffeine (Sowers and Galuska,1993, Lamke et al, 1976). A detailed contraceptive history was also taken to include methods used and duration of use in the older age group. Repeat measurements of BMD and anthropometry were taken every six months during the five year follow-up. A follow-up questionnaire was administered at each follow-up visit and collected similar information to the admission questionnaire. An interim analysis was made at two-and-a-half years.

Additional funding from the William and Flora Hewlett Foundation awarded towards the end of the study enabled a sub sample of women to have a total hip, spine and femoral neck measurement taken (n= 96). These women were all in the younger age group and

the measurement was taken on the same day or within 5-6 weeks of their last follow-up visit.

2.2 Recruitment procedure

All women were given an information sheet (Appendix 1) to read and asked to give written consent for participation in the study. Consent forms in Zulu and English can be found in Appendix 1.

The following numbers of women were required for the longitudinal study.

1. Women in the 15-19 age group; at least 100 in each of the four categories.
 - a. New users of depot medroxyprogesterone acetate (DMPA).
 - b. New users of norethisterone enanthate (NET-EN).
 - c. New users of combined oral contraceptives (COCs).
 - d. Women who had no history of hormonal contraceptive use and who were not current users.
2. Women in the 40-49 age group; at least 100 women in four categories:
 - a. Current users of depot medroxyprogesterone acetate (at least 1 year of use).
 - b. Current users of norethisterone enanthate (at least 1 year of use).
 - c. Current users of combined oral contraceptives (at least 1 year of use).
 - d. Women who had not used hormonal contraceptives for the past five years.

2.3 Criteria for selection of subjects

Women were screened prior to recruitment to ensure they were eligible to participate. A screening questionnaire was completed prior to recruitment (Appendix 2) using the following criteria:

Inclusion criteria:

1. 15-19 age group

- a. Aged between 15-19 years
- b. New acceptors of DMPA, NET-EN and COCs, and never users of hormonal contraceptives (control group).

2. 40-49 age group

- a. Aged between 40-49 years
- b. Women who were current users of DMPA, NET-EN and COCs with one year or more use, and women who currently did not use a hormonal method and who had not used hormonal contraceptives in the last 5 years.

Exclusion criteria:

1. Age less than 15 or more than 19 (15-19 age group), age less than 40 or more than 49 (40-49 age group)
2. Currently lactating or lactation that ended within six months.
3. Recent pregnancy (pregnancy that ended during the last six months).

4. Current or past medication of any drug for more than 3 months, known to affect calcium metabolism (anticonvulsants, corticosteroids; thyroid supplement; drugs to treat hyperthyroidism; vitamin D or calcium supplement; thiazide diuretics).
5. Chronic disease affecting bone metabolism (liver disease and diabetes mellitus) especially diseases known to affect calcium metabolism (chronic renal failure; hyperparathyroidism or hypoparathyroidism; hyperthyroidism or hypothyroidism; cancer; rickets, pituitary disease).
6. Hysterectomy or oophorectomy.
7. Bone mass 2 Standard deviations (SDs) below normal range.
8. Known to be HIV positive
9. Known to have menopausal (no menstrual cycle for at least 1 year)
- 10.. Indicators suggesting risk of HIV infection:-
 - Significant weight loss in last 6 months (5kg or more)
 - Chronic diarrhoea
 - Lymphadenopathy
 - Tuberculosis

Exclusion criteria during follow-up 15-19 & 40-49

1. Medication of any drug for more than 3 months, known to affect calcium metabolism (anticonvulsants, corticosteroids; thyroid supplement; drugs to treat hyperthyroidism; vitamin D or calcium supplement; thiazide diuretics).

2. Chronic disease affecting bone metabolism (liver disease and diabetes mellitus) especially diseases known to affect calcium metabolism (chronic renal failure; hyperparathyroidism or hypoparathyroidism; hyperthyroidism or hypothyroidism; cancer; rickets, pituitary disease).
3. Hysterectomy or oophorectomy.
4. Bone mass 2(SD) (Standard deviations) below normal range¹.
5. Known to be HIV positive
6. Pregnancy excluded women from the BMD measurement, however if women were willing to continue 6 months postpartum they could be continued in the study.

2.4 Admission procedure

Women who were eligible and volunteered to enter the study were taken through the informed consent process where written consent was sought prior to participation (Appendix 1). The admission interview took approximately thirty minutes to administer and was conducted in the client's home language by a trained research nurse employed by the Reproductive Health and HIV Research Unit (RHRU). (Appendix 2). Information collected included socioeconomic variables: education, employment and income; pregnancies and lactation history: current and past use of tobacco, alcohol and caffeine. A detailed contraceptive history was taken which included methods used and duration of use. If the participant was an existing client at the clinic, the client-retained family

¹ This was possible for the 40-49 age group where there are established reference populations available. The reference population in this study consisted of white European women and therefore was not directly comparable to a population of African women. There was no normal reference group available for the younger study group. The baseline data was used to compile a range for the population.

planning card was used to verify the contraceptive history taken. The medical examination included BMD measurement, blood pressure, anthropometry (height, weight, waist and hip) to assess health status. These results were recorded in the examination form (Appendix 2).

2.5 Bone mineral density measurements

Forearm BMD was measured by dual x-ray absorptiometry (DEXA). For each subject, two measures of forearm BMD density were made:- at the distal radius and at the midshaft of the forearm. At the distal end of the radius trabecular bone predominates, while the midshaft is mostly cortical in nature. In view of the differences in bone morphology at these sites, bone density measurement at two sites is useful and has a larger application.

Quality control for the bone measurements was achieved by ensuring that the research staff were thoroughly trained initially in the technique and repeated training thereafter.

The test/ retest reliability of the bone measurements was assessed during the pilot phase and on an on-going basis during the main phase of the study by repeating measurements taken during the examination. The principal investigator, who conducted the repeat measurements, did not have access to records of previous measurements. Repeat measurements (n=5) were conducted randomly on a bi-monthly basis. Daily phantom measurements were taken every morning on start-up of the equipment. Data from the phantom measurement was plotted daily to monitor measurement drift. Equipment was serviced yearly by a trained technician.

2.6 Visit Schedule

Study participants were interviewed by the research nurse at admission and scheduled for a follow-up visit every six- months. However if late for a scheduled follow-up or unable to attend, longer durations between follows ups were accepted in circumstances where as women were able to present family planning cards and give detailed contraceptive histories since last visit. At each follow-up visit all participants had repeat BMD and anthropometry measurements taken. Participants in the older age group had a blood test every year for follicle stimulating hormone (FSH) levels. Risk behaviour assessment was repeated at each six-monthly visit. Participants who discontinued their method continued to be followed-up for the five-year study period if they agreed to continue participation in the study.

2.7 Description of the DEXA equipment for BMD measurement

A number of techniques/ procedures are available to quantify BMD. These include radiogrammetry, single and dual photon/x-ray absorptiometry, computed tomography and total body calcium measurement by neutron activation analysis. The choice of the technique depends on several considerations, such as the site in the body selected for bone mass measurement (different body sites contain different ratios of trabecular/cortical bone), purpose of investigation (whether to identify individuals at risk of fracture, for diagnosis of a suspected metabolic disease, to monitor progress of a disease or therapy), level of precision and accuracy acceptable, cost of the

equipment/procedure, time required to carry out the procedure, and safety in terms of radiation exposure.

DEXA was chosen as the method for measuring bone mineral density in this study for several reasons. DEXA accurately reflects total body calcium, measures of bone mineral density using the method are highly reproducible: the co-efficient of variation is 1%. DEXA equipment is not expensive relative to other equipment used to measure bone mass and the method is easy to learn. Examinations using DEXA take about five minutes per subject to complete and so do not require a complicated procedure for the subject.

This study considered DEXA equipment that could measure central sites (spine, femoral neck and hip) however the cost of this equipment purchased exclusively for study use was prohibitive. The study further considered collaborations with academic departments to use their equipment however the amount of space required for the study and the volume of study participants seen daily would have been too disruptive for their routine appointment system. The cost per spine/hip scan in the private sector were also prohibitive for the study. The World Health Organisation (WHO) funded the study including the equipment. Funding for equipment covered the cost of new equipment similar to that used in a previous WHO BMD multi-centre study (Pettiti et al, 2000). In this study forearm BMD was measured in women aged 25-35 years in 7 centres internationally including Africa. A number of studies have shown that measurement of forearm bone mass is a good indicator of the bone mass situation at other sites (Need and Nordin, 1990, Duppe et al, 1997). The equipment purchased to measure BMD in this

study by dual photon/ x-ray absorptiometry was the DEXA model DTX-200 (Osteometer MediTech A/S Co, Rodovre, Denmark). The technique involved exposure to negligible radiation dose. The skin dose on the forearm was 0.2 mGy and the effective dose was 0.1 μ Sv per scan. The bone mineral content was expressed as mg/cm². The DEXA equipment was standardized daily using a phantom as prescribed by the manufacturer's instructions. Accuracy to the standard during the recruitment period was 0.53%. and in vivo precision was 0.94%.

An example of the forearm DEXA scan conducted in this study and information on the equipment can be found in Appendix 3.

The DEXA equipment used in the sub-study to measure hip, spine and femoral neck was a Hologic QDR discovery, version 12.6, Model: Discovery w(s/n) 49369. An example of this scan can be found in Appendix 3.

2.8 Menopausal status of women in the 40-49 age group

One of the challenges identified at the outset of the study was how to classify women by menopausal status. The injectable groups in particular required special consideration due to menstrual cycle irregularities. The sub-study around FSH levels (Chapter 3.8) confirmed that FSH levels were not masked by DMPA/NET-EN use outside of the peak suppression period. FSH levels were therefore used in this study as one of the criteria to indicate menopausal status.

Standard criteria for menopause can only be applied retrospectively. To consider a woman as having reached menopause, she should not have had any menstrual bleeding within the past 2 years. This criteria was adequate for the non-hormonal contraceptive user group. For injectable users this was not appropriate, therefore FSH levels were used in addition to bleeding patterns.

Women were classified as (a) normal, (b) peri-menopausal and (c) menopausal. This system was used yearly at the end of each following full year of follow-up.

At admission, menopause was an exclusion criteria. This was because the period of the study aimed to include the menopausal transition and those recruited could have potentially reached menopause at some point during the study follow-up. Women were accepted into the study if their menstruation was not regular. This was because it was anticipated that most women presenting for recruitment, in particular those aged 47-49, would have some menstrual irregularities and would be clinically defined as peri-menopausal.

Menopausal symptoms as indicators of menopause were asked at baseline and follow-up, however, hormone replacement therapy (HRT) treatment is not generally available in the public sector to most of these women so few women seek care. Table 2.1 presents the menopause classification system for the study.

Table 2.1 Menopause classification

Criteria	Normal	perimenopause	Menopausal
FSH level in last year (Miu/mL)	<20	>20 <25.8	25.8-134
*Bleeding pattern in last year	Regular cycle between 21 -35 days	Irregular bleeding cycle	No bleeding seen in past 2 years

*Only applicable to non-users of hormonal contraception

A woman in the non-user group must have had amenorrhea for two consecutive years before she was classified as fully menopausal. Women in the hormonal contraceptive groups (DMPA & NET-EN) must have had FSH levels in the menopausal range for two consecutive years and in addition have had no bleeding/spotting during this time. COC users who had FSH measured in the pill free cycle also used the same classification system as the injectable users. Their bleeding cycles would be artificially affected by their method.

Women enrolled in the 40-49 age group had a blood test taken at admission and every year to measure FSH levels. Women were also asked about any menopausal symptoms they may have experienced since the previous visit. Women with high FSH levels (above 30IU/l) and who exhibited vasomotor symptoms were referred to the clinic staff. Information on bleeding patterns, other menopausal symptoms and FSH/LH levels were monitored after discontinuation. If the FSH was still above 30 IU/L and there had been no bleeding for two years the woman was considered to be menopausal. All women discontinuing their method as a result of menopause were followed up for the full five years on the same six-monthly appointment schedule.

2.9 Sample Size

The proposed study aimed to be able to detect a half standard deviation difference in BMD between users and non-users of injectable contraceptives. This would be of biological significance as this difference would translate into a large difference in the risk of fracture in the older woman. Information on the mean and standard deviation of cross-sectional measurements of forearm bone mass made in white, European, premenopausal women that was reported by Nordin (1987) was used to estimate sample size. Data available on the association of bone mass with use of hormonal contraceptive has not always been consistent in showing an association in one direction, the sample size required for each category assuming a two-tailed statistical test with a probability value (alpha) of 0.05 and with a power (beta) of 0.80 was 63 subjects. Loss to follow up was estimated at 8-10% per year. Sample size was adjusted for this loss after consideration of potential levels of loss-to-follow-up rates in each age-group. It was anticipated that the younger woman may have higher rates of loss-to-follow-up compared to the older age-group, as this age group is more mobile and on completing their education may move home for education or work purposes. The sample size was increased to 110 as it was expected rates of loss-to-follow-up may be at least 10% per year. In addition it was expected that a proportion of the 15-19 year old non-users would commence on a method of contraception within the study period. To compensate for this the sample size for non-users was increased to 150.

It was anticipated that the older women would have loss to follow-up rates of approximately 8% per year and the sample size was adjusted to 100. We raised the

sample number of non-users in the 40-49 years age group as clinic records during the study preparation phase showed that this age group was not regular clinic attendees at the study site. These women were usually accompanying friends and relatives or attending for pap smears, and it was felt that loss-to-follow-up may be greater in this group compared to those who attended the clinic regularly for family planning supplies. The sample in this group was increased to 150

2.10 Statistical analysis

Multivariate analysis was performed to assess the effect of various factors, as well as to control for confounders. Since the data set contains repeat measures for each subject, it was necessary to take into account the within-subject correlation of observations. This was achieved by using generalised estimating equations (GEE) models or multi-level models as implemented by the 'xt' commands for panel data in the software package STATA (V.10 College station, TX, USA). The analysis had to use bone mass at various times as end products. Furthermore, subjects changing between contraceptive methods had to be taken into account by aggregating person years of exposure.

Multivariate adjustment was necessary in the analysis for several reasons. First, it was not possible to identify a comparison group of non-users of hormonal contraceptives who are identical to users of such methods in every way, except in their use of hormonal contraception, and even after exclusions, we expected some non-comparability between users and non-users of hormonal contraceptives. In the younger age group, only women

who were new users of either injectable or oral contraceptives were recruited, so eliminating the need to adjust for history of contraceptive use in this age group.

Linear regression was used to assess differences in bone mass between non-users of hormonal contraceptives and users of COCs and progestin-only injectable contraceptives, adjusting for differences between the groups on characteristics other than bone mass. Descriptive measures were used to assess variables such as past reproductive history, body mass, social characteristics and personal habits. An interim analysis was made after two-and-a-half years. BMD was measured in users and discontinuers.

Body mass index was calculated from the weight and height as kg/m^2 . BMD was measured in $\text{grams/centimetre}^2$ (g/cm^2) at two sites in the distal forearm (radius and ulna). The characteristics of women in the study were quantified as means $\pm$ SD, medians, or percentages. Differences in BMD between contraceptive groups, and the associations between BMD and selected characteristics of the study participants by contraceptive group were assessed using one way analysis of variance and multiple variable linear regression for BMD measured at the two anatomic sites.

Menopause was classified as amenorrhoea for at least one year. For injectable hormonal contraceptive users this criterion could not always be used as women commonly experienced amenorrhea induced by the method. Therefore in addition to amenorrhea, Follicle stimulating hormone (FSH) levels from blood samples were measured. An FSH level of ≥ 25.8 milli International units per milliliter (mIU/mL) was considered to be in

the menopausal range (King Edward VIII Hospital Durban; Chemical Pathology Laboratory criteria using Roche Elecsys FSH expected values). FSH levels were divided into two categories according to laboratory cut-off levels for premenopausal (<25.8 mIU/mL) and peri-menopausal/menopausal (≥ 25.8 mIU/mL).

Follow-up of all women was cumulated up to their last visit, or up to the visit preceding a visit at which they reported a change in contraceptive method, whichever occurred first. Interim analysis of this study found that BMI was significantly associated with radius BMD. To assess the effect of contraceptive method on radius BMD, and to allow for within subject correlation of responses whilst controlling for the confounding effect of changes in BMI, random effects linear regression methods were used with radius BMD at the time of a study visit as the response variable, and contraceptive method, duration of follow-up and BMI at time of the visit as explanatory variables. Differences in rate of change in radius BMD per year between methods were calculated by including interaction terms in the model for method and follow-up time. Additionally, we investigated whether change in radius BMD varied between two time periods by including an interaction term between follow-up time up to the last visit occurring before 2.5 years and follow-up time between 2.5 and 5 years, for each user-group separately. These two time periods were chosen as the literature indicates that the greatest loss of BMD as a result of hormonal contraceptive use occurs over the first two years of use (Tang et al, 2000) and then loss continues at a slower rate and appears to stabilize. Finally, we investigated BMD in women who discontinued hormonal contraception. Data

analysis commenced on completion of baseline data collection in 2002. Analysis of the main data-set commenced in 2007.

2.11 Data management

2.11.1 Data collection

Data were collected via questionnaire and clinical record form (Appendix 2). On the clinical record forms, the results of the measurements of height and weight, anthropometry and bone density value were recorded. Details of completion of questionnaires and coding can be found in Appendix 2.

3.11.2 Data entry

Questionnaires were collected from the site approximately every two weeks. Data were double entered by the trial assistants at the RHRU. The data entry programmes for all study instruments were written in Epi-info 6.04 (CDC, Atlanta). The data was routinely cleaned and checked by running frequencies and checking outliers in the dataset. Baseline data was finalized in 2003 in preparation for two publications. The data was imported into the STATA statistical analysis package for analysis (V.8- V10 College station, TX, USA). On completion of the study in May 2006, the final dataset was checked and cleaned and this process was completed by February 2007.

2.12 Duration of the study

2.12.1 Overview

The study was funded by the World Health organisation for six years (Jan 2000- April 2006). This enabled the women recruited in year one of the study to complete 5 years of follow-up. Those recruited in year 2 potentially were able to complete between 4 and 5 years of follow-up. The study had five phases; preparation (3 months); pilot (2 months); enrolment only (6 months); enrolment and follow-up (two years) and follow-up only (3 years). The main recruitment commenced in June 2000.

2.12.2 Preparation phase

This part of the study included gaining ethics approval, site preparation and planning, meeting with Department of Health staff to secure accommodation, training of staff in use of equipment and data collection, finalisation of the questionnaires and translation, writing of data entry programmes and purchasing supplies.

2.12.3 Pilot phase

The pilot phase tested the questionnaire, recruitment strategies, quality control procedures, rate of recruitment and acceptability of the measurement to clients.

2.12.4 Enrolment phase

It was anticipated that enrolment to complete all study groups would take in the region of 18-24 months. Some study groups were recruited in a shorter period.

2.13 Main problems experienced

NET-EN is the injectable product used by the majority of younger women and so recruitment of the DMPA and the COC group took longer in the younger age groups. The five-year follow-up required considerable effort to motivate the women to stay in the study for the full five years. At recruitment only women who were intending to live in the area for the next five years were enrolled. This was emphasised in the study information sheet and was discussed with the client during the screening interview. A trial assistant was employed to trace non-attendees.

2.14 Ethical considerations

Ethical approval was granted from the University of the Witwatersrand Human subjects Ethics Committee and the Scientific and Ethical Review Group (SERG) at the WHO (Appendix 4).

1. Women were asked to volunteer for the study
2. The study was explained in detail and an information sheet was available (Appendix 1).
3. Women were asked to sign a consent form (Appendix 1)
4. Participation involved an extra 30-45 minutes to the clinic consultation including the questionnaire administration and the physical measurements.
5. There was no pain or discomfort involved in the procedures involved.
6. The DEXA technique involved exposure to a negligible radiation dose.

7. All women received a stipend of R50.00 for each visit which was intended to cover any transport costs.

2.15 Study procedures

This following section provides comprehensive information on the study and its procedures. All aspects of the study and documentation used are recorded in this thesis. Additional information arising in the course of the study was added in the relevant section. Guidelines were developed to give further information on the conduct of this study. All staff involved in the study used these guidelines so that they were familiar with the aims, design and investigations of the research study.

2.15.1 General Principles

1. Each member of staff participating in the study was expected to be familiar with the protocol, not only with the part in which he or she was directly involved, but also with the other aspects of the study. In addition, all staff members were kept regularly informed about the study's progress.
2. It was essential that the study be carried out exactly as described in the protocol and that all data was recorded from all subjects. Incomplete observations and protocol violations would reduce the number of data suitable for analysis, and this could jeopardize achieving the study's objectives.

3. Women who volunteered to participate in this study were clearly informed about its purpose and about what their participation involved. The basic underlying object was to make participants aware that the contribution they were making was important and voluntary. Much of the information collected during the study was of a personal and sometimes sensitive nature. Health workers and other personnel involved in the study were expected to observe the rules of confidentiality and make it clear to women taking part that this was being done.

2.15.2 Recruitment

The study site was situated at the Commercial City family planning Clinic. This clinic is managed by the Department of Health and provides family planning, sexually transmitted infection (STI) management and counseling and referral for HIV testing and termination of pregnancy. It is located in the centre of Durban and is used mainly by women living, working and studying in and around the Durban city centre. It has easy access for transport and is open daily. At the start of the study the clinic was open on Saturday mornings, however in 2001 due to staff shortages the opening hours were restricted to week days. The clinic sees approximately 6-7,000 clients a month for family planning. The clinic is attended by mainly African woman but around 10-15% of clients are Indian or Coloured.

One consultation room in the clinic was given to the study for a period of six years. All screening, admission and follow-up procedures were conducted in this clinic. The research nurse and trial assistant were permanently based at the clinic at this time.

Flyers and posters informing clients about the study were displayed on the clinic walls and can be found in Appendix 5. For non-users of hormonal methods, flyers were folded and inserted into condom packets placed near the entrance of the clinic.

2.15.2.1 15-19 age group

Recruitment took place directly from the youth clinic waiting area. Clients normally reported directly to the reception clerk on entry to clinic and presented a client retained card. New clients had a card made for them. The reception clerk placed the cards in order of attendance time (no fixed appointments were made).

The research nurse checked cards for potential study age group criteria (15-19). If the client was in the correct age group, she would introduce herself to the client and explain the study. The nurse determined if the client could be screened whilst waiting for her appointment or after she has been seen by the clinic nurse, depending on how busy the clinic was at the time.

All new clients for family planning were seen by routine clinic staff for their initial assessment and prescription of method. Thereafter if recruited into the study at repeat visits, subjects could choose to be given their usual family planning method by the study nurse. This was an incentive for the participant as they did not need to wait in the normal clinic queue. The research nurse or study assistant took the client to the study consulting room for full screening followed by admission if eligibility was confirmed.

2.15.2.2 40-49 age group

Recruitment took place from the adult clinic. The receptionist at the adult clinic contacted the trial assistant if any woman in the 40-49 age group attended the clinic. The trial assistant would go to the adult clinic to explain the study. If the client was interested in taking part she was screened at the youth clinic. Non- users of hormonal contraception in this age group who were, attending for cervical smears, condoms, IUD checkups or accompanying friends and relatives were asked if they were interested in participating.

2.15.3 Screening

Potential candidates for the study completed the informed consent form and were screened by the research nurse in a private consulting room. If a woman was eligible she was informed in detail about the study procedures. If found to be ineligible for any reason, the screening form was kept for analysis.

2.15.4 Admission

Subjects admitted to the study completed the admission questionnaire and physical examination. Questionnaire details can be found in Appendix 1. Full procedures for examination are described in the following section:

2.15.4.1 Physical measurements

A number of measurements were taken during the admission visit. All these measurements were taken in privacy and other study staff members avoided entering the

room whilst measurements were being taken so as not to make the subject feel uncomfortable.

The measurements were taken in the following order:-

- Height
- Weight
- Waist/hip/arm circumference
- Blood pressure
- Blood test (40-49 age group) (FSH, LH)
- Bone mineral density measurement

Height

A scale fixed to a wall in the examination room was employed to measure height. After removing the shoes, the subject stood on a flat floor with feet parallel and with heels, buttocks, shoulder and back of head touching the upright. The head was held comfortably erect, with the lower border of the orbit (eye socket) in the same horizontal plane as the external auditory meatus. The arms were hanging at the sides in a natural manner. The bar was lowered making contact with the top of the head. Height was recorded to the nearest 0.1 of a centimeter.

Weight

The subjects stood on the centre of the platform wearing ordinary light clothing without touching anything else. Shoes were removed. Weight was recorded to the nearest 0.2 of a kilogram.

Circumferential measurements for assessment of adiposity

Waist circumference and hip circumference provide a readily measurable objective method for assessment of the type and degree of adiposity. When used along with the body mass index, these measurements provide a reliable method of documenting the degree of adiposity. All circumferential measurements were made with the non stretch study tape. The markings of the tape faced away from the skin of the subject and towards the observer.

The measurements were taken with the subject standing and waist exposed. Waist was measured just above the level of the lateral iliac crest, below the lowest rib, with the subject's arms hanging down passively. Hip circumference was measured just under the inferior rim of the symphysis at the midline. Circumferences were recorded to the nearest centimeter.

Arm circumference

To measure the arm circumference, the tape was placed gently, but firmly around the limb to avoid compression of the soft tissues. The midpoint of the upper arm was taken as halfway between the acromial process of the scapula and the olecranon process of the ulna. Measurements to the nearest 0.1 of a centimeter were recorded. The less dominant arm (i.e. left arm in right-handed subjects and right arm in left-handed subjects) was measured, while hanging freely, at its midpoint.

Blood pressure measurement

The subject was asked to remove their outer garments and expose the upper right arm for the blood pressure cuff. The arm should rest comfortably, palm up, on the desk. The investigator sat at the front of the desk close to the right-hand. The shirt sleeve was rolled or slid up to allow sufficient room to place the cuff. The shirt sleeve should not constrict the arm. If it did, the subject was asked to take her arm out of her shirt. The cuff was wrapped round the arm. The brachial pulse is located just medial to the biceps tendon and the cuff is positioned so that the centre of the inflation bag lay over the brachial artery. The lower edge should be 2 to 3 finger-breadths (about 2.5 cm) above the cubital fossa. The investigator felt the subject's radial pulse with one hand and keeping her hand on it, inflated the cuff slowly with her other hand until the radial pulse disappeared. This is the pulse obliteration pressure. The column of mercury was allowed to fall at 2mm per second. She listened for korotkoff sounds with the stethoscope on the brachial pulse, recorded systolic pressure (pressure at which first sound heard clearly) and phase v diastolic pressure (pressure at which sound disappears). These were recorded to the nearest 2mm Hg and the readings were recorded.

Bone Mineral Density Measurement

Forearm BMD measurement

The non-dominant arm (left in most cases) was used for measurement purposes. If the women indicated history of fracture in the non-dominant arm, the dominant arm was used.

The research nurse followed the following procedures:

- Completed the relevant details required for entry prior to the scan. Most of this information was collected prior to the measurement itself (age, height, weight etc)
- Asked the woman to roll up her sleeve past the elbow and remove her watch/bracelets etc. Rings were to be left in place.
- The arm was inserted into the scanner while the subject was seated comfortably next to the DEXA equipment and the hand held the central stem. The client was asked to find a comfortable seated position as they were required to keep the arm still for the duration of the measurement (4-5 minutes).
- On completion of the measurement the screen was checked to view the image of the forearm for the following important points:

- a Previous fracture unknown to the subject in the scanned area (area appeared dense and white and resulted in a raised measurement result).
- b. Movement of the subject could be detected by changes in smooth outline of forearm visible on the scan.
- c. Tissue area between bones on the scan was checked to see if any area was not represented by the red colour. In this case the cursor was used to indicate the correct areas on the scan and the information was entered.

Only when these major points had been considered was the scan accepted. If the scan was unclear or the participant moved the scan was repeated.

Spine/Hip/Femoral neck BMD measurement

The measurement was taken at Jackpersad, Rooknoodeen and partners Inc, Westridge Medical centre, Durban. This scan was taken by a qualified radiologist in the X-ray department. A study staff member was in attendance to welcome study participants and explained the process to them. The measurement was taken while the participant was lying flat on an examination couch and the measurement time was approximately 3 minutes.

Blood tests (40-49 age group)

Blood tests were taken at admission and yearly thereafter for measurement of FSH and LH.

The research nurse followed the following procedures:

Completed all the documentation and ensured that the blood tube was labeled with name, study number and project name. Any blood tubes collected were taken on the same day to Addington Hospital, chemical pathology laboratory for separation and storage. The trial assistant was responsible for daily transfer of samples between clinic and Addington Hospital.

3.15.4.2 Forms completed and information given at admission

The following forms were completed at admission:

- Admission
- Examination

- Contact details
- Lab form (40-49 age group)

Other administrative details

- FP card checked
- Follow-up appointment arranged

Verbal reminder of important points to subject

- Subjects were seen again after six months for full examination
- Subjects should come back for the appointment even if they change clinic / method.
- Subjects could phone study nurse at any time (phone number supplied).

After all the admission study procedures were completed, women using a hormonal method were given reminders of their repeat family planning visit 2/3 months later (depending on the hormonal method). They were given R50.00 compensation for study participation. At this and any following routine family planning visit, if the participant was seen by the study nurse for her contraceptive method she would be reminded of her next study visit.

2.15.5 Follow-up

2.15.5.1 General outline

Follow-up appointments were scheduled to be at approximately six-monthly intervals. An appointment slip with a return date was given to the woman or she could choose to have the date entered on her family planning card or she put the date in her diary. The window period for the six-month follow-up was 6-12 months after the follow-up

appointment date. No woman was seen earlier than 6 months. Women were seen before or after their routine family planning appointment depending on waiting times. Appointments were staggered to ensure least possible waiting times for subjects. Non-users of contraception who did not need to attend the clinic, were given appointments at six months intervals. Women were given R50.00 at each follow-up visit as compensation for study participation. This covered transport costs to the site. In cases where women moved out of the Durban area and still wished to continue in the study, arrangements were made to see women at a time when they were visiting Durban or the study paid additional travel costs.

2.15.5.2 Examinations

The same examinations and procedures were undertaken as at the admission visit:

- Height
- Weight
- Waist / hip / arm circumference
- Bone mass measurement
- Blood tests once a year for 40 – 49 age group (yearly at every other follow-up)

2.15.5.3 Forms completed and information given at follow-up visits

The following forms were completed at admission:-

- Examination
- Follow-up
- New contact forms if required

Verbal information given

- Motivation to continue with study
- Blood test results from test taken at last visit

2.15.6 Non-attendees

If a subject did not attend for her appointment on her stated return month, she would be contacted by telephone if she had consented and had given the study nurse her details on the confidential address form (Appendix 1). She would be reminded of her appointment and rebooked. Women in the study who did not have access to a telephone were sent reminder letters by post. If there was no response to either telephone calls or letters the trial assistant would visit the address and deliver a letter if the subject was not present. If the subject wished to discontinue, a discontinuation form was completed with details of why the subject withdrew from the study. All non-attendees' study cards were kept in a separate file until they had been contacted. If there was no contact with a subject for 18 months or the letter was returned undelivered the subject was discontinued as a loss-to-follow-up.

2.15.7 Blood test processing

Blood tests for measurement of FSH and LH were undertaken by:

Albert Luthuli Hospital, Dept of Chemical Pathology, Cato Manor, Durban

Initial processing of blood tests and storage were conducted by:

Addington Hospital, Dept of Chemical Pathology, Prince Street, Durban

Blood sample procedure:

All samples were labeled and transferred to Addington Hospital, Chemical Pathology on a daily basis. Samples were checked in, centrifuged and separated by lab staff and stored at -20°C. Depending on sample load, samples were taken every 2-4 weeks to Albert Luthuli for analysis. Test results were faxed to the Reproductive Health and HIV Research Unit within 2 days. Original test results were faxed or collected by a member of the study team.

2.15.8 Notification of results to subjects**2.15.8.1 Blood test results**

If the FSH / LH fell within normal levels, this was reported to participants at their next visit to clinic. If the level was raised (above 25.8 IU/ML), another test was taken at the next follow-up visit. If raised again the participant was referred to a clinician who would discuss appropriate changes in method.

2.15.8.2 Need for referral and referral criteria

There were instances where the subjects needed to be referred for further investigation, in these cases the following criteria were used:

- Subject was found to fall 2SD below limits set at the outset of the study for bone mass measurements.
- Subject requests procedure not undertaken by study, HIV test, sterilization or other medical treatment.
- Study nurse diagnoses a condition that requires referral e.g. high blood pressure.

- FSH/LH raised as per (2.15.8.1)

Standard referral letters were given to participants (Appendix 6)

The following referral mechanisms were agreed to with individuals and institutions.

- Subjects requested examination or test that is not part of the study. For example, the subject complained of symptoms of STIs. The study nurse will referred her to the routine clinic staff.
- Any conditions detected by the study nurse such as high blood pressure was referred to clinic staff.
- A clinician was available at the clinic on a sessional basis. Issues around menopause and test results related to menopause were referred to the clinic doctor (Dr Popis). The study nurse asked the subject to return during the doctor's sessions and provided the doctor with all necessary documentation that the study had collected in relation to the event in question.
- If the subject chose to be referred to her own local general practitioner a referral letter was be given to the subject to take with her.
- If the bone mass fell below 2SD of the normal range the client was referred for further investigation.

2.15.9 Quality Control

2.15.9.1 Bone mass measurement

Every two months a random sample of 5 women was re-measured. Measurements were conducted by a different investigator who did not have access to the original measurement.

Phantom measurement

Daily QC measurement was conducted by using the “phantom”. This is a perspex block representing the bone area measured in the forearm. This measurement should provide the same results on a daily basis which can be plotted to monitor measurement drift. The phantom block was inserted into the same position as the arm and a phantom scan was run on a daily basis as the first measurement of the day. The data generated by the phantom were checked daily to ensure it lay within expected limits. A monthly printout of phantom records were generated and filed in the QC file (Appendix 3). If the phantom reading showed signs of drift or any one measurement fell outside normal limits the technician from Marcus Medical was contacted immediately (Appendix 7 contact details). The equipment was serviced yearly throughout the study.

2.15.9.2 FSH / LH measurement

The laboratory had its own internal QC procedure. In addition every 20th blood sample drawn was duplicated. Extra blood was taken and an extra test was sent blind to the laboratory.

2.15.9.3 Physical measurements

Every two months a random sample of five women had a repeat height, weight, waist, hip, BP and arm circumference taken. Measurement was conducted by a different investigator who did not have access to original measurements.

2.15.9.4 Data forms

All forms were double-entered for verification, any discrepancies or queries were verified with the principal investigator

2.15.10 Collaborators

Details of collaborators and nature of collaboration can be found in Appendix 7.

CHAPTER 3.0 RESULTS

3.1 Organisation of the results

The results chapter is divided into seven sections and this first section briefly overviews the contents of each section. Section 3.2 describes the screening, recruitment and follow-up. Section 3.3 to 3.8 are made up of six papers (4 published, 1 in print and one in preparation) generated from the data collected during the study. The reprints of published work can be found in Appendix 8. Each paper contains its own methodology, results and discussion.

Section 3.3 presents the results of the analysis of the effect of hormonal contraception and BMD in the complete adolescent cohort follow-up. It also reports on the BMD of a small group of women who have discontinued NET-EN. This paper was published in May 2009.

Section 3.4 presents the results of a cross-sectional substudy, where hip, spine and femoral neck were measured. On completion of the five year cohort follow-up, a subsample of women (n=96) in the adolescent group participated in this component of the study. This paper will be published in August 09

Section 3.5 presents the results of the interim analysis based on the first 3 years of the study and includes the adolescent women. These women had been recruited between June 2000 and July 2002 and so by early 2004 women had been participating for between 18 months to 3 years. This interim analysis was published in 2007.

Section 3.6 presents cross-sectional baseline data from the older age group of women.

This paper was published in 2005 on completion of baseline data collection.

Section 3.7 presents the analysis of the longitudinal data in the older age group. This paper is in preparation and will be submitted in 2009.

Section 3.8 Presents the data from baseline analysis of the FSH and BMD in older woman using DMPA and NET-EN.

3.2 Screening, recruitment and follow-up

3.2.1 Screening and recruitment

Study recruitment commenced in June 2000. In total 1107 women were screened and 991 women were recruited. Five women were excluded shortly after recruitment and these exclusions were related to undisclosed medical conditions at screening (n=2) and women found to be outside the study age ranges (n=3) at screening. These 3 women had indicated they were in the correct age range at admission however all women were asked to bring in at the first follow-up visit (if not seen at admission) proof of age by means of an official document (ID book or family planning card). These 5 women were removed from the database. This resulted in a total of 986 eligible women continuing in the study.

The breakdown of the participants by study group is shown in the flow chart (Fig 1).

The minimum sample size for each group (100) was achieved across all groups. Groups

were over recruited where it was anticipated that there was potential for greater loss-to follow-up.

Figure 3.2.1 Number of women admitted into each study group

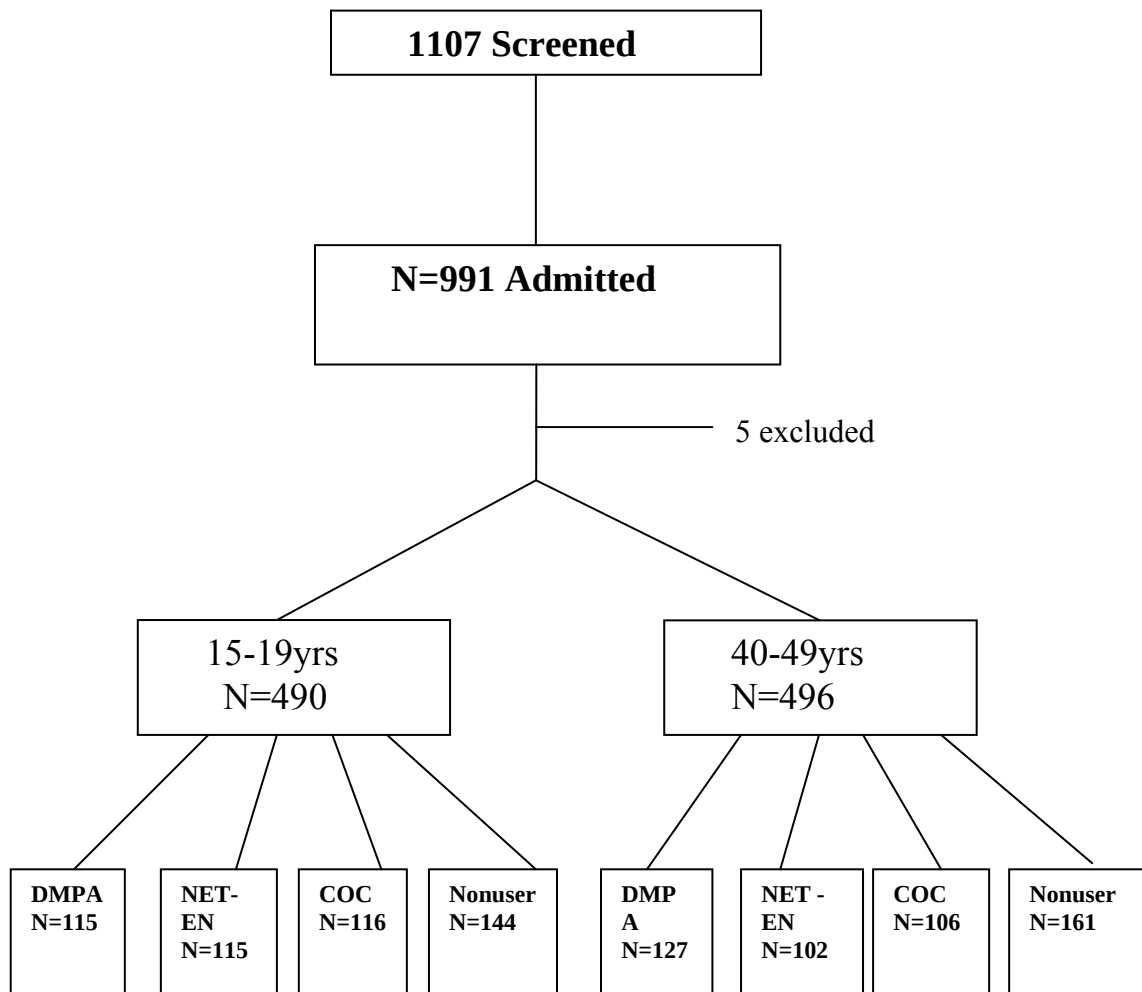


Table 3.2.1 shows the main reasons for exclusion at screening. In the older group many women were excluded due to existing medical conditions that could affect BMD. Some women had gynaecological histories that indicated that they may already have

menopausal. In the younger group women were mainly excluded for current or recent pregnancy and/or breastfeeding.

Table 3.2.1 Reasons for exclusion at screening

Exclusion criteria	Age group 15-19		Age group 40-49	
	%	(n=58)	%	(n=63)
Currently breastfeeding	8.6	(5)	14.2	(9)
Pregnant in last 6 months	51.7	(30)	3.1	(2)
TB	17.2	(10)	3.1	(2)
Out of age range	8.6	(5)	20.6	(13)
Hysterectomy/oophorectomy	0	(0)	9.5	(6)
menopause	N/A		7.9	(5)
Medical conditions*	3.7	(2)	41.2	(26)
Previous contraceptive use	10.3	(6)	N/A	

*Screening form lists medical conditions (Appendix 1)

Women were recruited and admitted from June 2000 until May 2003. Fifty five percent of women were recruited in the first year of the study and by the end of the second year all younger women had been recruited. The remaining 8% were recruited in year 3 and composed of older NET-EN users who were the most difficult category to recruit due to the low numbers of women who use this injectable product in this age group. The majority of women over 40 years of age and using injectable contraception use DMPA and the recruitment in this study group was completed within 18 months. In the younger group the slowest recruitment was in the DMPA group as NET-EN is the injectable method most popular with young women. This age group differential in use of the two available injectables persists across South Africa and has been well documented previously (Department of Health, South Africa, 2006).

3.2.2 Follow-up

The study continued to follow-up women until April 2006 when the study was completed. All women recruited in the first 10 months of the study were potentially able to participate in the full 5 years of follow-up. Those recruited after this time were followed up for a maximum of 3-4 years depending on their date of recruitment. Follow-up rates are shown in table 3.2.2 for both 2 and 4 years. It can be seen that the older age group follow-up rates are higher than those of the younger women.

Table 3.2.2 Follow-up rates at two and four years in each age group

Age group	2 years %	4 years* %
15-19	73	43
40-49	83	61

*Follow-up rate at four years is only shown for those women who potentially were able to complete at least 4 years in the study.

The study attempted to contact all women who did not attend their 6-monthly appointments. Women were contacted telephonically after they were one month late for their scheduled visit. If no telephone contact number was available a letter was sent as a reminder. After three letters had been sent a fieldworker attempted to visit the address given to locate the participant. If the participant had moved away they were discontinued from the study. A follow-up form was completed for non-attendees (Appendix 1), and those who were lost-to follow-up were discontinued and a discontinuation/end of study form was completed (Appendix 1). Women who were traced and still wished to participate were allowed to continue as long as they were able to document their contraceptive use and their family planning cards were checked.

Reasons for discontinuation before end of study are shown in Table 3.2.3. The main reasons for discontinuation was loss to follow-up and women moving out of the area. In cases of loss-to-follow-up the study staff were unable to contact the participant. Women who moved away often reported to the study that they would not be able to attend again. Eighteen women had died and the study was informed by a friend or

relative. Where available, cause of death was collected. Most of the deaths appeared to be AIDS related with several deaths due to TB.

Table 3.2.3 Reasons for study discontinuation

Reason for discontinuation	%	N=986
End of study (completed)	52.0	(513)
Loss to follow-up*	18.1	(179)
Pregnancy	3.0	(30)
Medical condition	1.9	(19)
Moved away**	22.1	(218)
Death	1.8	(18)
Withdrew	0.9	(9)
Total	100	986

* Loss to follow-up was defined as no contact after non-attendance

** Moved was defined as verbal or written contact with participant that she had moved out of the area and was no longer able to attend

3.3: Bone mineral density in a cohort of adolescents during use of norethisterone enanthate, depot-medroxyprogesterone acetate, or combined oral contraceptives and after discontinuation of norethisterone enanthate

Mags E Beksinska¹, Immo Kleinschmidt², Jenni A Smit¹, Timothy M M Farley³

This paper has been published in Contraception 2009; vol.79, no.5, pp.345-9

(Appendix 8)

¹Reproductive Health and HIV Research Unit, Department of Obstetrics and Gynaecology, University of the Witwatersrand, 3rd Floor, Westridge Medical Centre, 95 Jan Smuts Highway, Mayville, 4091, South Africa

²London School of Hygiene and Tropical Medicine, London UK.

³UNDP/UNFPA/WHO/World Bank Special Programme of Research, Development and Research Training in Human Reproduction, Department of Reproductive Health and Research, World Health Organization, Avenue Appia, CH 1211 Geneva 27, Switzerland.

Abstract

Background: Depot-medroxyprogesterone acetate (DMPA), norethisterone enanthate (NET-EN) and combined oral contraceptives (COCs) have been shown to have a negative effect on bone mineral density (BMD) in adolescents. The aim of this study was to investigate BMD in 15-to 19-year-old new users of DMPA, NET-EN and COCs.

Study Design: This 5-year longitudinal study followed-up new users of DMPA (n=115), NET-EN (n=115), and COCs (n=116), and 144 nonuser controls. BMD was measured at the distal radius using dual x-ray absorptiometry.

Results: BMD increased in all groups (annual percent increase: nonusers, 1.49%; DMPA, 1.39%; NET-EN, 1.03%; COCs, 0.84%) during follow-up ($p<0.001$). There was evidence for lower BMD increases per annum in NET-EN ($p=.050$) and COC ($p=.010$) users compared to nonusers but no difference between DMPA and nonusers ($p=.76$). In 14 NET-EN discontinuers, an overall reduction of 0.61% per year BMD was followed upon cessation by an increase of 0.69% per year ($p=.066$).

Conclusion: This study suggests that BMD increases in adolescents may be less in NET-EN and COC users; however, recovery of BMD in NET-EN users was found in the small sample of adolescents followed post-discontinuation.

Introduction

Depot-medroxyprogesterone acetate (DMPA) has been found to have a negative effect on bone mineral density (BMD) in adult premenopausal women (Curtis and Martins, 2006; Wanichsetakul et al, 2002; Petitti et al, 2000; Scholes et al, 1999;; Cundy et al, 1991) and in adolescents (Cromer et al, 2008; Clark et al, 2006; Scholes et al, 2005; Cromer et al, 2004; Lara-Torre et al, 2004; Scholes et al, 2004; Busen et al, 2003; Cromer et al, 1996). Limited data on the effect of norethisterone enanthate (NET-EN) have found a negative effect on BMD in adult (Rosenburg, et al, 2007) and adolescent users (Beksinska, et al 2007). A comprehensive review concluded that combined oral contraceptive (COC) use in adult premenopausal women was not associated with changes in BMD (Martins, et al, 2006). However, emerging data show that BMD may be compromised in adolescent users of low-dose COCs (Hartard, et al, 2006; Cromer, et al, 2004; Polatti et al, 1995).

Studies that have followed women after discontinuation of DMPA have found that BMD recovers in adult pre-menopausal women within approximately 2-3 years of cessation of the method (Kaunitz, et al, 2008; Scholes, et al, 2002). Two studies have followed-up adolescent users of DMPA post-discontinuation (Clark et al, 2006; Scholes et al, 2005), and in both these studies, a significant increase in BMD was found in adolescents who discontinued DMPA. No longitudinal data are available on recovery of BMD in adolescent NET-EN and low-dose COC users. More evidence is needed to show if the recovery of BMD found in adult users of hormonal contraception is replicated in adolescents. The objective of this study was to determine if long-term use of hormonal

contraceptives (COC, DMPA and NET-EN) compared to non-use, was associated with a change in bone mass in women aged 15-19 years.

Subjects and methods

This was a prospective longitudinal study of adolescents aged 15 to 19 years who had never used hormonal contraception prior to recruitment to the study. Initiators of DMPA, NET-EN, COCs, and nonusers of contraception were enrolled from a family planning clinic in Durban, South Africa. The study cohort was recruited between July 2000 and July 2002 and follow-up continued until April 2006. All DMPA users were started on a regimen of 150 mg every 12 weeks and NET-EN users on 200 mg every 8 weeks. Both DMPA and NET-EN was administered intramuscularly. The COCs used by women included a range of formulations, with almost all (93%) using low-dose formulations containing between 30-40 mcg of estrogen. Women were eligible to participate if they had not lactated or delivered in the past 6 months, were not currently or had never used medication known to affect calcium metabolism for more than 3 months, and did not have a chronic disease affecting calcium metabolism. At the baseline visit, participants' height, weight and blood pressure were measured using a standard protocol and a questionnaire was administered to elicit information on demographic characteristics, regularity of the menstrual cycle, smoking, diet, exercise and caffeine and alcohol intake.

Forearm BMD was measured by dual energy x-ray absorptionmetry (DXA model DTX-200, Osteometer MediTech A/S Co, Rodovre, Denmark). BMD was measured in grams per square centimetre (g/cm^2) at the distal radius in the forearm. The DXA equipment was

standardized daily using a phantom as prescribed by the manufacturer's instructions. Accuracy to the standard during the recruitment and follow-up period was 0.53%.

Study participants were followed-up at approximately six-monthly intervals. Forearm BMD, height and weight were measured at each follow-up visit. History of contraceptive use since last visit was recorded. Those recruited between July 2000 and April 2001 and who continued to participate until the end of the study completed 5 years of follow-up. Those recruited after April 2001 who continued until the end of the study, completed between 4 and 5 years of follow-up. Follow-up could not continue beyond April 2006 when project funding came to an end. Women continued the same follow-up schedule even if they stopped, changed or started another contraceptive method in order to investigate issues of recovery of BMD post-discontinuation of a hormonal method.

The characteristics of women in the study were quantified as means $\pm$ SD, medians, or percentages. The study was powered to detect a half standard deviation difference in bone mass between users and nonusers of hormonal contraceptives. This would be of biological significance as this difference is expected to translate into a large difference in the risk of fracture in older women. Information on the mean and standard deviation of cross-sectional measurements of forearm bone mass in white, European, premenopausal women reported by Nordin (Nordin, 1987) was used to estimate sample size. A sample of 63 participants per contraceptive group was required assuming a two-tailed statistical test with a significance level of 5% and power of 80%. Allowing for 10-15% loss to follow-up per year, this sample was increased to at least 110 women in each group to ensure

adequate numbers in long-term follow-up. The nonuser group was over recruited compared to other groups as, in addition to loss to follow-up, it was anticipated that some of these women would commence a method of contraception or become pregnant.

Differences in BMD between contraceptive groups, and the associations between BMD and selected characteristics of the study participants by contraceptive group were assessed using one-way analysis of variance and multiple variable linear regression. BMD was measured in users and discontinuers. Follow-up of all women was cumulated up to their last visit, or up to the visit preceding a visit at which they reported a change in contraceptive method, whichever occurred first.

Interim analysis of this study found that BMI was significantly associated with radius BMD (Beksinska, et al, 2007). To assess the effect of contraceptive method on radius BMD, and to allow for within subject correlation of responses whilst controlling for the confounding effect of changes in BMI, we used random effects linear regression methods with radius BMD at the time of a study visit as the response variable, and contraceptive method, duration of follow-up and BMI at time of the visit as explanatory variables (STATA V.10, College Station, TX, USA). Differences in rate of change in radius BMD per year between methods were calculated by including interaction terms in the model for method and follow-up time. Additionally, we investigated whether change in radius BMD varied between two time periods by including an interaction term between follow-up time up to the last visit occurring before 2.5 years and follow-up time between 2.5 and 5 years, for each user-group separately. These two time periods were chosen as the

literature indicates that the greatest loss of BMD as a result of hormonal contraceptive use occurs over the first 2 years of use (Tang,et al, 2000) and then loss continues at a slower rate and appears to stabilize. Finally, we investigated BMD in women who discontinued hormonal contraception.

Ethical approval was granted by the University of the Witwatersrand, Human Subjects Research Committee (protocol number M981001), and by the Scientific and Ethical Review Group of the World Health Organization (WHO).

Results

In total, 490 women aged 15-19 years were recruited. All women including those in the DMPA, NET-EN and COC groups had no past use of hormonal contraception before recruitment into the study. Baseline information about these women is summarized in Table 3.3.1. Mean age was just under 18 years and most women were still in full-time education. The majority of women were African except in the COC group, which included 22% women of Indian origin and 21% Coloured. More than a quarter of women (29.3%) in the DMPA user group had ever been pregnant compared to low prevalence of pregnancy in the other groups. COC users were more likely to be smokers than other method users, although smoking generally was low across all groups.

In total, 277 women continued with the same method of contraception for at least two follow-up visits, while the rest had changed method or stopped using contraception. Total same-method follow-up time was 648 person-years in these 277 participants. Women

who continued in the study using a different method of contraception, or stopped using contraception, were included up to the visit they changed method. Overall, 64% of woman continued in the study for between 2-3 years and 43% completed between 4-5 years of follow-up depending on time of recruitment. Main reasons for discontinuation from the study included pregnancy, illness that could have affected BMD such as tuberculosis, loss to follow-up and moving out of the area.

Table 3.3.1 Socio-demographic, lifestyle and reproductive characteristics of subjects aged 15-19 years by contraceptive user group, at baseline.

Characteristics	DMPA (n=115)	NET-EN (n=115)	COC (n=116)	Non user controls (n=144)	P value
Mean age, years (SD)	17.8 (1.4)	17.4 (1.3)	17.8 (1.0)	17.4 (1.2)	0.0002
Mean highest education grade (SD)	10.9 (1.5)	10.5 (1.5)	11.3(0.8)	10.3 (1.4)	0.0001
Marital status, %					
Married/cohabiting	5.3	5.2	10.3	12.8	0.0001
Reg partner-not cohabiting	92.1	83.8	87.1	63.5	
Casual partner	2.6	5.2	2.6	23.0	
No partner	0	0.9	0	0.7	
Employment status %					
Employed full/part-time	6.2	2.6	9.5	2.7	<0.0001
Unemployed	11.4	10.5	8.5	7.4	
Student/scholar	82.5	86.8	79.1	98.8	
Ethnicity %					<0.0001
African	94	89	57	92	
Coloured	5	7	21	4	
Indian	1	4	22	4	
Ever pregnant %	29.3	3	6	2	<0.0001
Ever lactated %	16.7	0.9	2.6	1.4	
Lactation (yrs) median	<1	<1	<1	<1	0.19
Mean age at menarche, years(SD)	13.9±1.3	13.8±1.2	13.6±1.48	13.5±1.29	
Exercise					
No regular exercise (%)	78.1	72.8	64.9	77.7	0.0001
At least once a week (%)	21.9	27.2	35.1	22.3	
Dieted in last 6 months (%)	3.5	2.6	5.3	2.0	0.54
Ever smoked cigarettes (%)	9.6	11.3	27.3	4.8	< 0.001
Current smoker (%)	6.0	7.0	13.9	3.4	0.006

DMPA= depot medroxyprogesterone acetate; NET-EN = Norethisterone enanthate;
COC= combined oral contraceptive SD= standard deviation;

Table 3.3.2 shows mean BMD and BMI at enrolment for each group. Radius BMD was similar in all four contraceptive user groups at baseline (p=.196). The regression model

showed that BMI was the only baseline characteristic associated with radius BMD ($p < 0.001$). BMI increased consistently by 0.11 kg/m^2 per year (95% CI 0.04 to 0.18) in all user groups with no evidence of differences in growth between groups ($p = 0.14$).

Table 3.3.2 Mean BMD and BMI at admission by contraceptive group

Variable	DMPA n=115	NET-EN n=115	COC n=116	Nonuser n=144	p value
Radius BMD g/cm^2	0.459 (0.06)	0.446 (0.06)	0.460 (0.05)	0.455(0.05)	0.196
Body weight (kg)	59.1 (12.1)	59.5 (9.8)	56.7 (11.4)	59.7 (11.6)	0.13
Height (cm)	154.8 (5.2)	156.1 (5.7)	156.2 (7.5)	155.9 (7.3)	0.30
BMI (kg/m^2)	24.63 (4.77)	24.31 (3.75)	23.18 (4.97)	24.61 (5.85)	0.097

BMD= bone mineral density; BMI=body mass index

Data are expressed as mean $\pm$ SD

Radius BMD increased in all groups during follow-up (Table 3.3.3), even after adjusting for changes in BMI ($p < .001$) with an overall increase for all women combined of 1.39% per annum (95% CI 1.19 % to 1.59 %). In nonusers, BMD increased by 1.49% per annum; in the DMPA, NET-EN and COC users, the increases were less 1.39% ($p = .76$), 1.03 % ($p = .05$) and 0.84% ($p = .01$), respectively (Table 3.4.3). There was moderate to strong evidence for smaller increases per annum in NET-EN ($p = .050$) and COC ($p = .010$) users compared to nonusers. There was no evidence for a difference in BMD between DMPA and nonusers ($p = .76$) (Table 3.3.3).

Table 3.3.3 Factors associated with radius BMD at study visit, estimated from random effects liner regression model.

Variable	% change in BMD (95% CI) per annum	% change in BMD per method relative to non user (p)	N	Time at risk (years)
Use of contraceptive method, per year*				
Non user (reference)	1.49 (1.25-1.72)	0	96	311
DMPA,	1.39 (0.79-1.98)	-0.10 (p=0.76)	51	76
NET-EN	1.03 (0.63-1.44)	-0.45 (p=0.050)	71	146
COC	0.84 (0.39-1.28)	-0.65(p=0.010)	59	116
Effect of BMI, per unit change in BMI (% per kg/m ²) [†]	0.267 (0.157-0.377)		277	648

*Adjusted for BMI

[†] Adjusted for contraceptive method

There was no evidence of a difference in change in radius BMD in the two time periods (up to 2.5 years and 2.5 years and longer) in any of the four study groups (DMPA p=0.105, NET-EN p=0.7, nonusers p=0.46, COC p=0.06).

We investigated changes in BMD in a group of 14 NET-EN users who discontinued use of the method without restarting another method during the course of the study. An overall reduction in radius BMD of 0.61% (95% CI= -2.53% to 1.34%) per year was seen in the 14 NET-EN users during use of the method, followed upon cessation of method by an increase of 0.69% (95% CI= -0.18% to 1.56%) per year. After adjusting for BMI, there was some evidence of a difference in rate of BMD change pre and post use of NET-EN (p=0.066). Most COC and DMPA discontinuers restarted other methods, thus there were too few to conduct a group analysis.

Discussion

Our results show that radius BMD increased during follow-up in the nonuser and all three user groups after adjusting for BMI. There was some evidence of less increase in NET-EN and COC groups compared to the nonuser group. Our results confirm those obtained from an interim analysis of this study (Beksinska, et al, 2007) in regard to NET-EN. However, in the preliminary analysis, COC users did not show any differences in BMD growth from nonusers, possibly because the interim analysis lacked power. In the only other study that has previously investigated the effect of NET-EN use (Rosenburg, et al, 2007), BMD was measured in the left calcaneus in a large cross-sectional sample of adult premenopausal women. NET-EN users had similar values to DMPA users and these were both significantly lower compared to never users.

We found no evidence of any change in BMD growth in the period up to 2.5 years of use compared to the period from 2.5 years of use. Several studies have reported that the rate of loss of BMD in DMPA users is greatest in the first two years of use (Clark, et al, 2006; Curtis and Martins, 2006; Scholes, et al, 2005; Tang, et al, 2000). Only one of these studies has investigated rate of change in adolescents (Scholes, et al, 2005).

Several longitudinal studies have found that adolescent users of low-dose COCs may gain bone mass at a slower rate compared to nonusers (Hartard et al, 2006; Cromer, et al 2004; Polatti, et al, 1995). The COC formulations in our study were similar to those reported in these studies. Our study also showed that COC users gained BMD at a slower rate than nonusers. Although DMPA users had a lower yearly increase in BMD compared to

nonusers, this was not significant. Other studies measuring BMD at central sites such as the hip and spine have found that adolescent DMPA users have lower BMD values compared to nonusers (Cromer et al, 2008; Clark et al, 2006; Scholes et al, 2005; Cromer et al, 2004; Lara-Torre et al, 2004; Scholes et al, 2004; Busen et al, 2003; Cromer et al, 1996). Studies using the forearm as a site of BMD measurement have generally not found significant differences between DMPA users and controls (Bahmondes, et al, 1999; Tharnprisarn, et al, 2002). Our study has found similar results to others measuring the forearm with respect to DMPA. The forearm may be more sensitive to the effect of NET-EN and COC and it may be that these results would be magnified if measured at a central site such as the hip or spine. No studies have measured BMD at central sites in NET-EN users.

Our study shows some evidence of a recovery in growth in BMD in a very small sample of NET-EN discontinuers who did not use another method of contraception after cessation. The cross-sectional study that investigated women who commenced NET-EN during adolescence (18 years and younger) and then ceased using the method (Rosenburg, et al, 2007), found no difference in BMD values between those who had ceased use 2-3 years before and never users regardless of whether they were early starters (adolescents) or late starters (post-adolescence). It was not possible to make any statement about BMD recovery in the COC and DMPA discontinuers in our study as numbers were so small.

DMPA suppresses estradiol production, leading to estrogen deficiency (Ortiz, et al, 1977). This leads to greater bone resorption and hence loss of BMD. There is less information on NET-EN and the mechanisms by which it could affect BMD. Studies on NET alone have not found negative effects on BMD (Horowitz, et al, 1993; Eldred, et al, 1992; Riis, et al, 1990). There is now limited data available on recovery of BMD in women who commence DMPA in adolescence (Clark, et al, 2006; Scholes, et al, 2005). This is the first longitudinal study which has included NET-EN users and discontinuers. Further evidence is needed to ensure that any losses of BMD as a result of DMPA and NET-EN in adolescence are fully recovered and peak bone mass is not compromised. Until that time, the recommendations for DMPA and NET-EN in adolescents will continue to caution long-term use in young women.

The third edition (2004) of the World Health Organization's Medical Eligibility Criteria for contraceptive Use (WHO, 2004) has changed the classification of use of DMPA and NET-EN for women below 18 years and above 45 years from category 1 (no restriction for the use of the contraceptive method), to category 2 (advantages of using the method generally outweigh the theoretical or proven risks). This is backed up in the document by a number of statements reporting on current evidence of the effect of DMPA on BMD. The WHO has recommended that in the absence of evidence on NET-EN, the same restrictions should apply to NET-EN users.

Our study shows that although BMD continues to increase in adolescent users of NET-EN and COCs, these increases are lower than in nonusers. Although the percent yearly

increase in DMPA users was lower than nonusers, there was no evidence of a difference and this may have been because this group had the lowest number of years at risk included in the analysis.

Limitations

Longitudinal studies of adolescent users of hormonal contraception are often limited by method discontinuation. This was the case in our study where few women remained on hormonal contraception for more than two years without a change of method or stopping use of contraception altogether. Concerns regarding long-term use of DMPA and NET-EN in adolescents should take this into consideration as many young women will have discontinued use before the 2-year review suggested on the patient labelling. Our study has used the forearm as its site of measurement which may be less sensitive than the hip and spine to changes in BMD resulting from hormonal contraceptive use.

3.4 Bone mineral density in young women aged 19-24 after 4-5 years of exclusive and mixed use of hormonal contraception

Mags E Beksinska¹, Immo Kleinschmidt², Jenni A Smit¹ Timothy M M Farley³

This paper is in press at *Contraception* 2009; vol.79, no.8 (Appendix 8)

¹Reproductive Health and HIV Research Unit, Department of Obstetrics and Gynaecology, University of the Witwatersrand, Mayville, 4091, South Africa

²London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E

³UNDP/UNFPA/WHO/World Bank Special Programme of Research, Development and Research Training in Human Reproduction, CH 1211 Geneva 27, Switzerland.

Abstract

Background: Use of Depot-medroxyprogesterone acetate (DMPA), norethisterone enanthate (NET-EN) and low-dose combined oral contraceptives (COCs) has been associated with loss of bone mineral density (BMD) in adolescents. However, the effect of using a combination of these methods over time in this age-group is limited. The aim of this cross-sectional study was to investigate BMD in young women (aged 19-24 years) with a history of mixed hormonal contraceptive use.

Study design: BMD was measured at the spine, hip and femoral neck using dual X-ray absorptiometry. Women were classified into 3 groups; 1) injectable users (DMPA, NET-EN or both) (n=40), 2) Mixed COC and injectable users (n=13), and 3) nonuser control. (n=41).

Results: Women in the injectables only user group were found to have lower BMDs compared to the nonuser group at all three sites and there was evidence of a difference in BMD between these two groups at the spine after adjusting for BMI ($p=0.042$), hip ($p=0.025$), and femoral neck ($p=0.023$). The mixed COC/injectable user group BMD values were lower than controls; however, there was no evidence of a significant difference between this group and the nonuser group at any of the three sites.

Conclusion: This study suggests that BMD is lower in long-term injectable users, but not when women have mixed injectable and COC use.

Introduction

The long-acting progestogen injectable contraceptives depot-medroxyprogesterone acetate (DMPA) and norethisterone enanthate (NET-EN) have been found to have a negative effect on bone mineral density (BMD) in adult premenopausal women (Curtis and Martins, 2006; Wanichsetakul et al, 2002; Petitti et al, 2000; Scholes et al, 1999; Cundy et al, 1991) and in adolescents (Beksinska, et al, 2009; Cromer et al, 2008; Rosenburg, et al, 2007; Clark et al, 2006; Scholes et al, 2005; Cromer et al, 2004; Lara-Torre et al, 2004; Scholes et al, 2004; Busen et al, 2003; Cromer et al, 1996). The effect of combined oral contraceptives (COCs) on BMD has been found to be variable with no effect reported in adult premenopausal women (Martins, et al, 2006) but growing evidence that low-dose COCs may be detrimental to BMD in adolescents and young women (Hartard, et al, 2006; Cromer, et al, 2004; Polatti, et al, 1995).

Although recovery of BMD following discontinuation of DMPA is documented in adult pre-menopausal women (Kaunitz, et al, 2006; Scholes, et al, 2002) and in adolescent users (Clark, et al, 2006; Scholes, et al, 2005), concerns regarding bone loss in DMPA users resulted in an US Food and Drug Administration Black Box warning for DMPA stating its use may impair BMD (FDA, 2004). In the UK, the Medicines and Healthcare Products Regulatory Board (MHRA) suggest that another contraceptive method be used after 2 years of DMPA use (FFPRHC, 2004). Much less information is available on recovery in NET-EN users as it is not as widely used as DMPA and not available in the US or UK. Limited data have found recovery of BMD in adolescent NET-EN users (Beksinska, et al, 2009; Rosenburg, et al, 2007).

More evidence is needed on long-term use of hormonal contraception and, in particular, on women who switch from hormonal injectables to another hormonal method of contraception. The objective of this study was to determine if long-term use of hormonal contraceptives, including mixed use (COCs and/or, DMPA and/or NET-EN), was associated with a change in bone mass in women aged 19-24 years compared to nonusers.

Subjects and methods

This cross-sectional study of young women aged 19-24 years measured BMD in the hip, spine and femoral neck. All the women had been recruited 4-5 years previously into a longitudinal study where the BMD in the distal radius had been measured at 6-month intervals. Details of the longitudinal study methodology and results are described elsewhere (Beksinska, et al, 2009). At the end of the follow-up period, additional funds allowed for 100 single measurements of the central BMD sites providing an opportunity for measurements at other sites. A subsample of women attending their final longitudinal follow-up visit were informed about this additional component of the study at their last cohort visit and were invited to participate. This subsample included all women completing the study over a 5-month period.

At the start of the 5-year longitudinal study, initiators of DMPA, NET-EN, COCs and nonusers of contraception were enrolled from a family planning clinic in Durban, South Africa. All women recruited had no history of hormonal contraceptive use prior to enrolment (n=490). The study cohort was recruited between July 2000 and July 2002, and

follow-up continued until April 2006. All DMPA users were started on a regimen of 150 mg every 12 weeks and NET-EN users on 200 mg every 8 weeks. Both DMPA and NET-EN was administered intramuscularly. The COCs used by women included a range of formulations, with almost all (93%) using low-dose formulations containing between 30-40 mcg of estrogen. Women were eligible to participate if they had not lactated or delivered in the past 6 months; were not currently using and had not used medication known to affect calcium metabolism for more than 3 months; and did not have a chronic disease affecting calcium metabolism. At the baseline and follow-up visits, participants' height, weight and blood pressure were measured using a standard protocol and a questionnaire was administered to elicit information on demographic characteristics, regularity of the menstrual cycle, smoking, diet, exercise and caffeine and alcohol intake. It was from this cohort that the 100 women were recruited for the cross-sectional study reported here.

History of contraceptive use had been recorded in the longitudinal study over the total follow-up period at approximately 6-monthly intervals. Women in the cohort continued the same follow-up schedule even if they stopped, changed or started a contraceptive method. The additional visit to measure the hip, spine and femoral neck BMD was conducted between Oct 2005 and Feb 2006. The DXA equipment used in the cross-sectional substudy to measure hip, spine and femoral neck was a Hologic QDR discovery, version 12.6, Model: Discovery w(s/n) 49369, Hologic. Inc, USA. The DXA equipment was standardized daily using a phantom and accuracy to the standard during the measurement phase was 0.44%.

The characteristics of women in the study were quantified as means $\pm$ SD, medians, or percentages. Differences in BMD and weight between contraceptive groups, and the associations between spine, hip and femoral neck BMD and contraceptive group were assessed using one-way analysis of variance and multiple variable linear regression. Data were analyzed using the statistical package STATA V.10, (College Station, TX, USA).

Ethical approval was granted by the University of the Witwatersrand, Human Subjects Research Committee (protocol number M981001), and by the Scientific and Ethical Review Group of the World Health Organization.

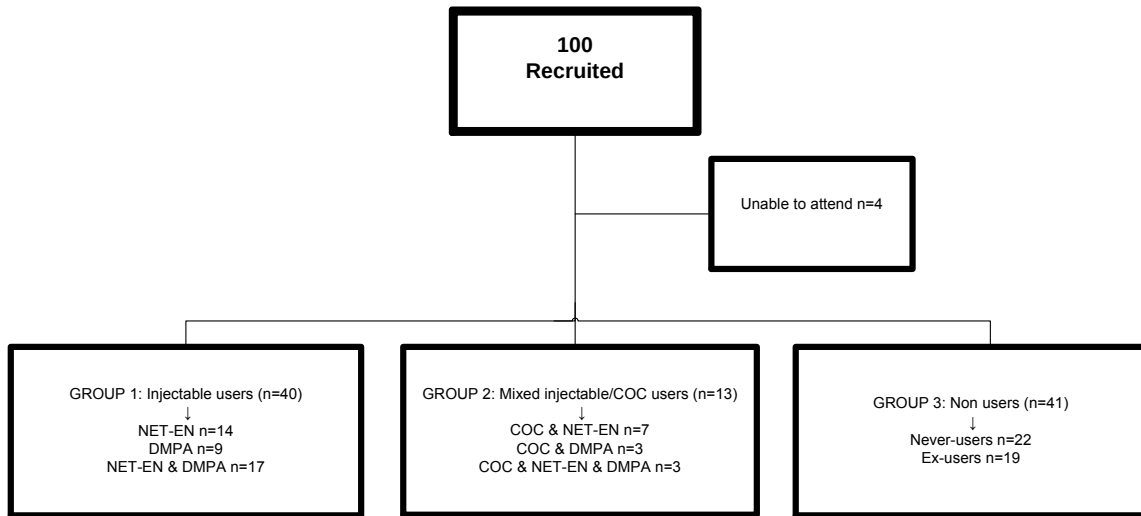
Results

A group of 100 women who had completed follow-up in the longitudinal phase agreed to participate in the cross-sectional substudy. Of these, 96 women attended their appointment and 4 women were unable to attend due to work commitments. The contraceptive history of these women varied considerably as women had not been discontinued from the longitudinal study if they changed or stopped a method. Thirty women presented with a history of mixed contraceptive use, including women who had used at least two of the study methods (DMPA, NET-EN, and COCs) in the preceding 4-5 years. Of these 30, 17 women had used both DMPA and NET-EN, 10 had mixed COCs and either DMPA or NET-EN, and three women had used all three methods. Far fewer women presented as exclusive users of one method at the end of the follow-up period

(DMPA n=9, NET-EN n=14, COC users n=2). The remaining 41 women were nonusers of hormonal contraception.

The small number of women who had used one method only, made data difficult to analyse and we therefore classified the women into three broad groups for analysis, according to their contraceptive history over the previous 4-5 years in the longitudinal component of the study. The first group (n=40) included all users of injectable hormonal contraception, regardless of whether they had used only one or both of the injection types. The second user group comprised of women who mixed COCs with injections (n=13). Finally, the non-hormonal contraceptive users (n=41) included 22 never users of hormonal contraception and 19 ex-users. The ex-users had not used a hormonal method for approximately 2 years or longer and almost all had less than 1 year of lifetime use. These women had started a method on recruitment into the longitudinal study and had subsequently discontinued use. We excluded the two exclusive COC users as they could not be classified into any of the three groups. Fig. 3.5.1 shows details of the three groups.

Fig 1. Participant Contraceptive history by study analysis group



Note: 2 COC exclusive users excluded

Baseline information about these women is summarized in Table 3.4.1. Mean age was approximately 22 years in all groups and the majority of women were African. Few women participated in any regular exercise and only one woman was a current smoker. Alcohol consumption was low across all groups.

Table 3.4.1 Characteristics of subjects aged 19-24 years by contraceptive user group

Characteristics	Injectable Users (n=40)	Mixed COC and injectable users (n=13)	Nonusers (n=41)	P value
Mean age, years (SD)	22.3 (1.4)	22.1 (1.2)	22.0(1.6)	0.7
Ethnicity %				
African	90.0	100.0	97.6	0.5
Coloured	7.5	0	2.4	
Indian	2.5	0	0	
Ever pregnant %	12.5	0	4.8	0.23
Mean age at menarche, years(SD)	13.9 (1.5)	13.8 (1.2)	13.7 (1.3)	0.47
Exercise > once a week (%)	5.1	16.7	5.4	0.05
Dieted in last 6 months (%)	2.5	0	0	0.52
Current smoker (%)	0.0	0	2.6	0.51
Alcohol consumption, units per week %				
Never	92.5	100.0	73.2	0.17
1-2 units	7.5	0	22.0	
3-4 units	0	0	4.9	

SD=standard deviation

There was evidence that BMD was associated with BMI for all three sites, with one unit of BMI corresponding to an average increase in BMD of 0.010 (0.006-0.015), 0.014 (0.009-0.017) and 0.017 (0.012-0.021) g/cm² for spine, hip and femoral neck, respectively.

Table 3.4.2 shows mean BMD at the three sites measured. Women in the injectables-only user group were found to have lower BMDs compared to the nonuser group at all three sites. There was some evidence of a significant difference in BMD between the

injectable and nonuser groups at the spine (p=0.042), hip (p=0.025), and femoral neck (p=0.023) after adjusting for BMI. In the mixed COC and injectable group, the BMD values were lower than the nonusers at all sites; however, there was no evidence of a difference between this group and the nonusers (spine p=0.54, hip p=0.15, femoral neck p=0.43).

Table 3.4.2 Mean spine, hip and femoral neck BMD by contraceptive user group, relative to nonusers

	Injectable users (n=40)	Mixed COC and injectable users (n=13)	Nonusers (n=41)
MeanBMDg/cm ² (SD)			
Spine	0.944 (0.109)(p=0.042)	0.960(0.094) (P= 0.54)	0.975 (0.115)
Hip	0.928 (0.116)(p=0.025)	0.919 (0.099) (P=0.15)	0.954 (0.102)
femoral neck	0.844 (0.125)(p=0.023)	0.855(0.101) (P= 0.43)	0.871 (0.130)
Mean BMI (kg/m ²) (SD)	26.4 (4.9) (p=0.14)	25.4 (3.1) (p= 0.71)	24.9 (4.3)

BMD=bone mineral density; BMI=body mass index; p-values from regression model adjusting for BMI

Weight in the nonuser group remained similar to that at baseline, whilst women in the injectable user group had gained on average 6.7 kg and the mixed COC/injectable group gained 1.9 kg (data not shown) over the 4-5 year follow-up.

Discussion

Our results show evidence of significantly lower BMD values in the hip, spine and femoral neck, between the adolescent users of injectable hormonal methods and nonusers, but not those women who have used COCs in combination with injectables. The absence of a significant difference in BMD between this group and the nonusers, may be due to the small number in this group (n=13). Over the follow-up period, this mixed-use group used COCs before and after injectable use and some moved between

these methods several times. However, this group had less overall injectable exposure over the follow-up period compared to the injectable-only group.

Although we are unable to comment on any specific method, our sample consisted of a representative group of women completing follow-up in the longitudinal study during a specific time period. At the end of our study, the mixed hormonal contraceptive users were found to be far more common than the exclusive users. Few women stayed on one hormonal method and many had discontinued hormonal contraception altogether. Although the study was designed to collect reasons for discontinuation of method, anecdotal reports indicated that various side effects and change in relationship status resulted in breaks and changes in method.

The literature has shown that discontinuation rates amongst adolescent DMPA users are particularly high, and continuation rates as low as 27% at one year have been found in the US (Polaneczky and Liblanc, 1998). In the longitudinal component of our study, there was an incidence of 37% method change per person-year for DMPA and 26% for NET-EN (Beksinska, et al, 2007). Although concerns remain regarding hormonal contraceptive use in adolescents, data indicate that few women are long-term exclusive users of one method of contraception. If mixed users are more prevalent, as was seen in our study, it would be important to include them in studies looking at hormonal contraceptive use and BMD.

Two studies have looked at young women who commenced low-dose COCs after use of DMPA and found they were at risk for loss of BMD (Berenson, et al, 2008; Albertazzi, et al, 2006). Our study could not confirm this finding as our numbers using DMPA followed by COCs was too small. In addition, the ethinyl estradiol (EE) formulations used by the COC users in our study was 30-40 mcg whereas studies that have found a detrimental effect of COCs on BMD have used even lower (20 mcg) EE formulations (Berenson, et al, 2008; Hartard, et al, 2006; Cromer, et al, 2004; Polatti, et al, 1995). The Medicines and Healthcare Product Regulatory Agency, UK (MHRA) guidelines (FFPRHC, 2004) recommend changing from DMPA to another method of contraception after 2 years of use; however, there is no advice as to what would be the most suitable method post-discontinuation. As the body of evidence increases on recovery of BMD in adult and adolescent women post discontinuation of DMPA (Kaunitz, 2008), this recommendation may need to be reviewed and balanced against the benefit of using DMPA as a long-term effective contraceptive. The third edition of the WHO Medical Eligibility Criteria for contraceptive Use (WHO, 2004) states the advantages of using DMPA generally outweigh the theoretical or proven risks in relation to bone mineral density. A position paper published in 2006 by the Society of Adolescent Medicine (Society for Adolescent Medicine, 2006) supports the WHO position on DMPA for woman below 18 and above 45 years and states that with existing evidence, the advantages of using DMPA in women below 18 years outweigh the disadvantages.

Limited data on recovery of BMD on cessation of hormonal contraceptive use in adolescents have been reported in exclusive users of DMPA (Clark, et al 2006; Scholes,

et al, 2005) and NET-EN (Rosenburg, et al, 2007; Beksinska, et al, 2009). Further evidence is needed to show that any loss of BMD, resulting from hormonal contraceptive use in adolescence, are fully recovered, and peak bone mass is not compromised. Until this time, the recommendations for DMPA and NET-EN use in adolescents will continue to caution long-term use in young women.

Limitations

This study was unable to analyze the individual effects of any of the three hormonal methods used as the sample size of exclusive users was too small for analysis.

3.5 Bone mineral density in adolescents using norethisterone enanthate, depot-medroxyprogesterone acetate, or combined oral contraceptives for contraception

Mags E Beksinska¹, Immo Kleinschmidt², Jenni A Smit¹, Timothy M M Farley³

This paper has been published in *Contraception* 2007; 75 (6) ;438-443. (Appendix 8)

¹Reproductive Health and HIV Research Unit, Department of Obstetrics and Gynaecology, University of the Witwatersrand, 3rd Floor, Westridge Medical Center, 95 Jan Smuts Highway, Mayville, 4091, South Africa

²Medical Research Council, 491 Ridge Road, Durban, 4091, South Africa

³UNDP/UNFPA/WHO/World Bank Special Programme of Research, Development and Research Training in Human Reproduction, Department of Reproductive Health and Research, World Health Organization, Avenue Appia, CH 1211 Geneva 27, Switzerland.

Abstract

Purpose: Most studies have shown a negative effect of depot-medroxyprogesterone acetate (DMPA) on the bone mineral density (BMD) of adolescents. There is no information available on the effect of norethisterone enanthate (NET-EN) on BMD in adolescents and the effect of combined oral contraceptives (COCs) on adolescent BMD is inconclusive. The aim of this longitudinal study was to investigate BMD in adolescent (aged 15-19 years) new users of hormonal contraception (DMPA, NET-EN and COCs).

Method: New users of DMPA (n=115), NET-EN (n=115), COCs (n=116) and 144 nonuser controls were recruited. BMD was measured at the distal radius and midshaft of the ulna using dual x-ray absorptiometry.

Results: In total, 275 women were included in this interim analysis and total follow up time was 553 person years. There was no significant difference in radius BMD between users of different contraceptive methods at baseline, $p=0.40$. Overall, an increase in radius BMD of 0.00522 per person year was observed. This result was similar when adjusting for BMI in the random effects regression model ($p=0.88$). The regression model showed that BMI was significantly associated with radius BMD, with each unit increase in BMI corresponding to an increase of 0.0029 g/cm² in BMD [95% CI 0.0023 to 0.0036, $p<0.001$]. Interaction between contraceptive method and follow-up time adjusted for BMI, was not significant ($p=0.07$). The increase in BMD for NET-EN users of 0.0013 g/cm² per person year [95% CI -0.0017 to 0.0043] was significantly lower than that of non users ($p=0.017$). For DMPA and COC users the increase in BMD was not significantly different compared to the nonusers. This study suggests that NET-EN users had lower increase in BMD over time compared to the other user groups.

Introduction

Studies undertaken to investigate the effect of hormonal contraception on bone mineral density (BMD) have mostly found that depot-medroxyprogesterone acetate (DMPA) has a negative effect on bone mass (Curtis and Martins, 2006; Wanichsetakul et al, 2002; Petitti et al, 2000; Scholes et al, 1999; Cundy et al, 1991), although some have shown minimal or no effect (Bahamodes et al, 1999; Gbolade et al, 1998; Taneepanichskul et al, 1997). There is limited information available on the effect of norethisterone enanthate (NET-EN) on BMD (Beksinska et al, 2005). The evidence for the effect of combined oral contraceptives (COCs) in premenopausal women has shown that in general COCs are not associated with changes in BMD (Martins et al, 2006; Paoletti et al, 2004; Nappi et al, 2003).

A number of studies have investigated the effect of hormonal contraceptives on BMD in adolescents aged less than 20 years (Scholes et al, 2005; Cromer et al, 2004; Lara-Torre et al, 2004; Scholes et al, 2004; Busen et al, 2003; Tharnprisan et al, 2002; Cromer et al, 1996) and almost all of these have found that DMPA has an adverse effect on BMD (Scholes et al, 2005; Cromer et al, 2004; Lara-Torre et al, 2004; Scholes et al, 2004; Busen et al, 2003; Cromer et al, 1996). No studies have looked at adolescent NET-EN users. Emerging data in young COC users are showing that BMD may be compromised in users of low-dose COCs (Hartard et al, 2006; Martins et al, 2006; Cromer et al, 2004). Studies of adolescent users of hormonal contraception have often been limited by small sample sizes (Busen et al, 2003; Cromer et al, 1996) and follow-up in longitudinal studies

on adolescents is often poor due to method discontinuation (Lara-Torre et al, 2004; Busen et al, 2003),

DMPA and NET-EN are effective contraceptives with low failure rates (WHO, 1983). In South Africa use of both hormonal injectable contraceptives is high amongst adolescents, with about 70% of Black African women aged 15-19 years practising contraception using either DMPA or NET-EN (DOH, 2006). Adolescence is a period of rapid growth and women gain 40-50% of their skeletal mass during this time (Sabatier et al, 1996). Loss of bone mass as a result of hormonal contraceptive use may have more serious implications in these women who have yet to achieve peak bone mass. It would be important to know if using hormonal methods of contraception interferes with the process of bone mineralisation. The objective of the study was to determine whether long-term use of hormonal contraceptives (COCs, DMPA and NET-EN) compared to non-use, is associated with a change in bone mass in women aged 15-19 years.

Subjects and Methods

A cohort of adolescent never-users of hormonal contraception aged 15 to 19 years starting use of DMPA, NET-EN, COCs, and nonusers of contraception were recruited from a large family planning clinic in Durban, South Africa. All eligible women were informed about the study and invited to participate if they wished. Recruitment commenced in July 2000 and was completed in 2002. For inclusion into the study women had to have no history of hormonal contraceptive use. All DMPA users were started on a regimen of 150mg every 12 weeks and NET-EN users on 200mg every 8

weeks. Both DMPA and NET-EN were administered intramuscularly. The COCs used by women included a range of formulations, with most (93%) using formulations containing between 30-40 mcg of oestrogen. Women were eligible to participate if they had not lactated or delivered in the past 6 months; were not currently or had never used medication known to affect calcium metabolism for more than 3 months; and did not have a chronic disease affecting calcium metabolism. On recruitment, a questionnaire was administered by a trained research nurse to elicit information on lifetime contraceptive history, fertility history and regularity of the menstrual cycle. Questions were also asked on smoking, diet, exercise and caffeine and alcohol intake. The examination included height, weight and blood pressure using a standard protocol.

Body mass index (BMI) was calculated from the weight and height as kilograms per square metre (kg/m^2). Forearm BMD was measured by dual energy x-ray absorptionmetry (DXA model DTX-200, Osteometer MediTech A/S Co, Rodovre, Denmark). BMD was measured in grams per square centimetre (g/cm^2) at two distal sites in the forearm (radius and ulna). The distal site contains approximately 25% trabecular bone and 75% cortical bone. The DXA equipment was standardized daily using a phantom as per manufacturers instructions. Accuracy to the standard during the recruitment and follow-up period was 0.53%. and in vivo precision was 0.94%.

Study participants are being followed-up at six-monthly intervals for a total of 5 years. Forearm BMD, height and weight were measured at each follow-up visit. The findings presented in this paper are based on an interim analysis and data collected up to the end

of 2004 are included. Those recruited early in the study had completed over 3 years follow-up, however those recruited in 2002 have approximately 18 months follow-up. Women were not excluded from the study if they stopped, changed or started a contraceptive method. This was done to investigate issues of recovery of BMD post discontinuation of a hormonal method. The method of contraception used was recorded at each follow-up visit. Women were assigned to the contraceptive method they were using until their next follow-up visit. If at any visit a change was recorded, the woman would be assigned according to the new contraceptive method. Thus, each woman could contribute to more than one of the exposure categories. This interim analysis is conducted on all women who remained on the same method for at least two visits inclusive of the baseline visit (minimum six months) and data are included up to the time the method was changed.

Ethical approval was granted by the University of the Witwatersrand, Human Subjects Research Committee, and by the Scientific and Ethical Review Group of the World Health Organization (WHO).

The characteristics of women in the study were quantified as means $\pm$ SD, medians, or percentages. The study was powered to be able to detect a half standard deviation difference in bone mass between users and nonusers of hormonal contraceptives. This could be of biological significance as this difference would translate into a large difference in the risk of fracture in the older woman. Information on the mean

and standard deviation of cross-sectional measurements of forearm bone mass in white, European, premenopausal women reported by Nordin (Nordin, 1987) was used to estimate sample size. A sample of 110 participants per contraceptive group was required assuming a two-tailed statistical test with a significance level of 5%, power of 80%, and allowing for 10-15% loss to follow-up per year. The nonuser group was over recruited compared to other groups as in addition to loss to follow-up it was anticipated that some women would commence a method of contraception or become pregnant.

Differences in BMD between contraceptive groups, and the associations between BMD and selected characteristics of the study participants by contraceptive group were assessed using one way analysis of variance and multiple variable linear regression. Follow-up of all women was cumulated up to their last visit, or up to the visit preceding a visit at which they reported a change in contraceptive method, whichever occurred first. To assess the effect of contraceptive method on radius BMD, and to control for the confounding effect of changes in BMI, a random effects linear regression model was set up using STATA (V.8 College station, TX, USA) with radius BMD at the time of a study visit as the response variable, and contraceptive method, follow-up since last study visit and BMI at time of the visit as explanatory variables, and allowing for within-subject correlation of responses. Differences in rate of change in radius BMD per year between method were assessed by including interaction terms in the model for method and follow-up time.

Results

In total, 490 women aged 15-19 were recruited. All women including those in the DMPA, NET-EN and COC groups had no past use of hormonal contraception before recruitment into the study. Baseline information about these women is summarised in Table 3.6.1. Mean age was between 17 and 18 years with the DMPA and COC groups being 4 months older than the nonuser and NET-EN groups ($p=0.0002$). Almost all women were African except in the COC group which included about a quarter (22%) of Indian women. This resulted in a significant difference in ethnicity between the groups. Smoking was low across all groups.

Table 3.5.1 Socio-demographic, lifestyle and reproductive characteristics of subjects aged 15-19 years by contraceptive user group, at baseline.

Characteristics	DMPA (n=115)	NET-EN (n=115)	COC (n=116)	Non user controls (n=144)	P value
Mean age, years (SD)	17.8 (1.4)	17.4 (1.3)	17.8 (1.0)	17.4 (1.2)	0.0002
Mean highest education grade (SD)	10.9 (1.5)	10.5 (1.5)	11.3(0.8)	10.3 (1.4)	0.0001
Marital status, %					
Married/cohabiting	5.3	5.2	10.3	12.8	0.0001
Reg partner-not cohabiting	92.1	83.8	87.1	63.5	
Casual partner	2.6	5.2	2.6	23.0	
No partner	0	0.9	0	0.7	
Employment status %					
Employed full/part-time	6.2	2.6	9.5	2.7	<0.0001
Unemployed	11.4	10.5	8.5	7.4	
Student/scholar	82.5	86.8	79.1	98.8	
Ethnicity %					<0.0001
African	94	89	57	92	
Coloured	5	7	21	4	
Indian	1	4	22	4	
Ever pregnant %	29.3	3	6	2	<0.0001
Ever lactated %	16.7	0.9	2.6	1.4	
Lactation (yrs) median	<1	<1	<1	<1	
Mean age at menarche, years(SD)	13.9±1.3	13.8±1.2	13.6±1.48	13.5±1.29	0.19
Exercise					
No regular exercise (%)	78.1	72.8	64.9	77.7	0.0001
at least once a week (%)	21.9	27.2	35.1	22.3	
Dieted in last 6 months (%)	3.5	2.6	5.3	2.0	0.54
Ever smoked cigarettes (%)	9.6	11.3	27.3	4.8	>0.00001
Current smoker (%)	6	7.0	13.9	3.4	0.006

DMPA= depot medroxyprogesterone acetate; NET-EN = Norethisterone enanthate;
COC= combined oral contraceptive; SD= standard deviation.

The reproductive characteristics of the woman are summarised in Table 3.5.1. Few women had ever been pregnant (teenage pregnancies) except in the DMPA group with a

quarter of women (29%) reporting that they were ever pregnant. Age of menarche was similar in all groups and at recruitment all had regular menstrual cycles, between 21 and 35 days duration (data not shown).

Table 3.5.2 shows mean BMD at both sites and BMI at enrolment for each group. There was no significant difference in radius ($p=.196$) and ulna ($p=.0534$) BMD between the four contraceptive user groups at baseline. The COC group had a slightly lower BMI at baseline but this was not significant.

Table 3.5.2 Mean BMD and BMI at admission by contraceptive group

	DMPA n=115	NET-EN n=115	COC n=116	Nonuser n=144	p value
Radius BMD g/cm ² (SD)	0.459(0.06)	0.446(0.06)	0.460(0.05)	0.455(0.05)	0.196
Ulna BMD g/cm ² (SD)	0.443(0.06)	0.432(0.06)	0.429(0.05)	0.436(0.06)	0.053
Mean BMI (kg/m ²) (SD)	24.6 (4.77)	24.3 (3.75)	23.2 (4.9)	24.6 (5.8)	0.097

BMI=body mass index

Table 3.5.3 shows the baseline BMD of women by age at enrolment. The increase in Radius and Ulna BMD of approximately 3% per year of age was significant.

Table 3.5.3 Mean BMD and BMI by year of age in 15-19 age group at admission

	15 n=36	16 n=57	17 n=101	18 n=173	19 n=123	P value
Radius g/cm ² (SD)	0.412 (0.049)	0.443 (0.054)	0.457 (0.053)	0.455 (0.52)	0.472 (0.056)	<.00001
Ulna g/cm ² (SD)	0.405 (0.054)	0.419 (0.053)	0.440 (0.053)	0.436 (0.053)	0.452 (0.062)	<.00001
Mean BMI (kg/m ²) (SD)	23.6 (4.3)	23.0 (3.5)	24.2 (4.8)	24.3 (5.6)	24.9 (4.9)	0.169

In this interim analysis period, 401 women had attended at least once for follow-up (82%). Of these women, 31 presented with criteria that made them ineligible to continue in the study. Pregnancy was the main reason for discontinuation and this was high in the nonuser control group. In total, 76% (n=370) women completed their second visit. Main reasons for discontinuation from the study included pregnancy, illness that could have affected BMD e.g. Tuberculosis, and moving out of the area. Of those who continued participation in the study 50% changed, stopped or started a method at least once during their follow-up. Some women (15%) changed, stopped or started a new method at least twice. Incidence of method change per year is shown in Table 3.5.4. Reasons given for method change were mainly change in relationship status or complaints about side effects of the method. This was lowest in the nonuser group where 19% started a method of contraception during their follow-up and highest in the DMPA group where 74% changed or stopped use. DMPA users often changed to NET-EN which is the more popular choice amongst young women.

Table 3.5.4 Radius BMD at baseline, follow-up time and incidence of method change by contraceptive group

Method	N	Follow-up time in person yrs	Incidence of method change per person year	Radius BMD g/cm ² (unadjusted) at baseline (SD)	p value
DMPA	51	75.42	0.37	0.46 (0.06)	0.40
NET-EN	68	125.68	0.26	0.44 (0.06)	
Non user	96	248.65	0.004	0.45 (0.05)	
COC	60	103.67	0.19	0.46 (0.05)	
Total	275	553			

In total, 275 women had continued the same method for at six months (two visits). Some women had contributed 7 study visits (approximately 3.5 years) in the study. Total follow up time was 553 person years in 275 participants (Table 3.5.4).

Overall, an increase in radius BMD of 0.00522 per person year was observed. Mean BMD of the women in this analysis was 0.45 g/cm² (SD=0.053) at baseline. There was no significant difference in radius BMD between prospective users of different contraceptive methods at baseline, $p=0.40$ (Table 3.5.4). This result was similar when adjusting for BMI in the random effects regression model ($p=0.88$). The regression model showed that BMI was significantly associated with radius BMD, with each unit increase in BMI corresponding to an increase of 0.0029 g/cm² in BMD [95% CI 0.0023 to 0.0036, $p<0.001$] (Table 3.5.5).

Table 3.5.5. Factors associated with radius BMD at study visit, estimated from the random effects liner regression model.

Variable	Effect on radius BMD	95% Confidence interval	p
BMI, change in radius BMD per unit change in BMI (g/ cm ² per kg/m ²)	0.0029	0.0023 to 0.0036	<0.001
Method relative to nonuser (g/cm ²)	0		
Non user (reference)	0.0058	-0.011 to 0.022	0.69
DMPA,	-0.0022	-0.017 to 0.013	0.77
NET-EN	0.0026	-0.013 to 0.018	0.74
COC			
Change in radius BMD over time (g/cm ² per person year) [†]			
Non user	0.0055	0.0037 to 0.0073	<0.001
DMPA,	0.0069	0.0028 to 0.011	0.001
NET-EN	0.0013	-0.0017 to 0.0043	0.40
COC	0.0053	0.0022 to 0.0085	0.001

Interaction effect of change in radius BMD over time and contraceptive user groups was marginally significant, $p=0.07$.

[†]In a model without BMI, interaction between method and follow-up time was significant with $p=0.036$.

Interaction between contraceptive method and follow-up time adjusted for BMI, was marginally non-significant overall ($p=0.07$). For DMPA users, the increase in radius BMD of 0.0069 g/cm^2 per person year [95% CI 0.0028 to 0.011] was non-significantly different compared to the increase of 0.0055 g/cm^2 per person year [95% CI 0.0037 to 0.0073] for nonusers ($p=.55$). Similarly the increase of 0.0053 g/cm^2 per person year [95% CI 0.0022 to 0.0085] for COC users was non-significantly different from the controls. The increase in BMD for NET-EN users of 0.0013 g/cm^2 per person year [95% CI -0.0017 to 0.0043] was significantly lower than that of non users ($p= .017$), and not inconsistent with the null hypothesis of no increase in BMD over time for these women ($p=0.40$).

Discussion

Women in the 15-19 age group have yet to reach peak bone mass and our baseline data indicates that there is a small yearly increase of approximately 2-3% per year in a population that had no exposure to hormonal methods of contraception on recruitment into the study. This increase would be expected in woman in this age range (Sabatier, et al, 1996) and our data indicate that forearm BMD is sensitive enough to document this increase.

The multiple variable analysis shows that BMD is strongly related to BMI ($p<0.001$). BMD in the non user reference group, the DMPA user group and the COC users group increased significantly during follow-up, by similar amounts (0.0055, 0.0069 and

0.0053g/cm² per year respectively) after adjusting for BMI. By contrast, the NET-EN group differed significantly from the nonuser group (p=0.017), achieving no significant growth in BMD during follow-up. This is the first study to date that has investigated the effect of NET-EN on BMD. NET-EN was the only group where there was no significant increase in BMD found over time (p=.4). No significant difference was found between either the COC and the nonuser control group or the DMPA and the nonuser control group.

Our study has used the forearm as its site of measurement and it may be that differences in the lumbar spine and hip are more sensitive and any decreases are magnified at these sites. Other studies (Tharnprisan et al, 2002; Bahamodes et al, 1999) using the forearm as the site of measurement found no significant differences between DMPA and controls. Studies finding decreases in BMD in users of DMPA have mainly used the hip and spine for measurement of BMD.

The loss of BMD in the case of DMPA has been associated with estrogen deficiency (Ortiz et al, 1977). Some studies show that these negative changes may be reversible and there is a return to pre-use levels on discontinuation of DMPA (Kaunitz et al, 2006; Petitti et al, 2000). In addition, there seems to be recovery of BMD in long term users of DMPA (Petitti et al, 2000; Taneepanichskul et al, 1997). However there is no long term data on women who commence DMPA in adolescence and therefore there is a need to investigate whether the recovery seen in older age groups applies to this younger group whose BMD may be compromised prior to achieving peak bone mass. It has been

suggested that norethisterone (NET) on its own may protect against calcium loss via a direct topic effect on bone or by the action of estrogenic metabolites.

The studies that have included NET (Horowitz et al, 1993; Eldred et al, 1992; Riis et al, 1990) have shown a positive effect on BMD; however, NET-EN may be different in that the depot characteristics are added to this compound through the addition of the enanthate (EN). The lack of data on the effect of NET-EN on BMD may be because the method is not as widely used internationally as DMPA. However, in South Africa this method has increased in popularity in recent years and is often the method of choice for younger women (DOH, 2006; Smit et al, 2001).

In 2004, the US FDA required the addition of a black box warning stating that DMPA may impair BMD (FDA, 2004) and adolescents were mentioned as a group that could be particularly at risk of bone loss. The following year the WHO issued a statement on hormonal contraception and bone health (WHO, 2006). Recommendations with regard to bone metabolism are that there should be no restriction on the use of DMPA and its duration of use in women below 18 and above 45 years. Evidence around potential safety concerns for women under 18 years using DMPA was not considered adequate at the time and therefore the WHO stated that the advantages of using DMPA outweighed the concerns regarding fracture risk. Recommendations for DMPA applied to NET-EN use. No restriction was placed on use of other progestogen-only or combined hormonal contraceptive methods. A position paper published in 2006 by the society of adolescent medicine (Society of Adolescent Medicine, 2006) supports the WHO position on DMPA

for woman below 18 years and above 45 years and states that with existing evidence, the advantages of using DMPA in women below 18 years outweigh the disadvantages. The lack of evidence on the consequences of long-term use of DMPA was acknowledged and until data became available, it was recommended that prescribing DMPA to adolescents should be considered on a case-by-case basis. A recently published paper (Albertazzi et al, 2006) has cautioned against the recommendation made by the UK Medicines and Healthcare Products Regulatory Agency (MHRA) (FFPRHC, 2004) that DMPA users should switch to another method of contraception after two years of use. Data in the Albertazzi et al paper (Albertazzi et al, 2006) shows that women using COCs after DMPA may be particularly at risk to loss of BMD. This present study suggests that DMPA and COCs do not affect BMD in younger women, but raises questions about the effect of NET-EN on BMD in younger users.

Future analysis on completion of our study will include information from a larger sample with longer follow-up and may clarify the effect of NET-EN further. In addition we will be able to examine potential recovery in those who stop using hormonal contraceptive methods. A sub sample of women in our study will have a hip and spine BMD measurements, but these data are not yet available.

The main limitations to our study were that long-term follow-up in our adolescent women has been affected by frequent method change and discontinuation. Our study has used the forearm as its site of measurement which may be less sensitive than the hip and spine to changes in BMD resulting from hormonal contraceptive use.

3.6 Bone mineral density in women aged 40-49 years using depot-medroxyprogesterone acetate, norethisterone enanthate or combined oral contraceptives for contraception.

Mags E Beksinska¹, Jenni A Smit¹, Immo Kleinschmidt², Timothy MM Farley³,
Fikile Mbatha¹,

This paper has been published in *Contraception* 2005; vol.71, no.3, pp.170-5.

(Appendix 8)

¹The Reproductive Health and HIV Research Unit, Department of Obstetrics and Gynaecology, University of the Witwatersrand, South Africa, Suite 1301, Maritime House, 143 Salmon Grove, Durban, South Africa.

²Medical Research Council, 491 Ridge Road, Durban, 4019, South Africa.

³Special Programme for Research, Development and Research Training in Human Reproduction, Department of Reproductive Health and Research, World Health Organization, CH-1211 Geneva 27, Switzerland.

Abstract

Background: Most studies show that depot-medroxyprogesterone acetate (DMPA) has a negative effect on bone mass. There are conflicting reports with respect to recovery of bone mass in long-term use of DMPA. No information is available on the effect of norethisterone enanthate (NET-EN) on bone mass, and combined oral contraceptives (COCs) have not been found to be associated with loss of bone mass. The aim of this study was to investigate bone mineral density (BMD) in older women (40-49 years) in relation to use of DMPA, NET-EN and COCs for at least 12 months preceding recruitment into the study.

Study Design: One hundred and twenty seven users of DMPA, 102 NET-EN users and 106 COC users were compared to 161 nonuser controls. BMD was measured at the distal radius and midshaft of the ulna using dual X-ray absorptiometry.

Results: There was no significant difference in BMD between the four contraceptive user groups ($p=0.26$) with and without adjustment for age. Although a small decrease in BMD was noted in the age range 40 to 49 years this was not statistically significant ($p=0.7$). The BMD was found to be significantly associated with body mass index (BMI) ($p<0.0001$) at both measurement sites, with an increase of one unit of BMI translating to an increase of 0.0044 g/cm^2 in radius BMD. Follicle stimulating hormone (FSH) level $\geq 25.8 \text{ mIU/mL}$ was associated with a decrease of 0.017 g/cm^2 in radius BMD relative to women with FSH $<25.8 \text{ mIU/mL}$. Significant interaction between FSH and BMI in their effect on BMD was observed ($p=.006$). This study found no evidence that long-term use of DMPA, NET-EN and COCs affects BMD in this population.

Introduction

Cross-sectional studies undertaken to investigate the effect of hormonal contraceptives on bone mineral density (BMD) have produced conflicting reports (Wanichsetakul et al, 2002; Petitti et al, 2000; Bahamondes et al, 1999; Scholes et al, 1999; Taneepanichskul et al, 1997; Cundy et al, 1996; Cundy et al, 1991). Most have indicated that depot-medroxyprogesterone acetate (DMPA) has a negative effect on bone mass (Wanichsetakul et al, 2002; Petitti et al, 2000; Scholes et al, 1999; Cundy et al, 1996; Cundy et al, 1991), and some have shown minimal or no effect (Gbolade et al, 1998; Bahamondes et al, 1997; Taneepanichskul et al, 1997). The loss of BMD in the case of DMPA has been associated with estrogen deficiency (Ortiz et al, 1977). Some of these studies show that these negative changes are reversible and there is a return to pre-use levels on discontinuation of the method (Petitti, et al, 2000; Orr-Walker et al, 1998). In addition there has been evidence of recovery of BMD in long-term users (Petitti et al, 2000; Tang et al, 2000; Taneepanichskul et al, 1997). There is no information available on the effect of norethisterone enanthate (NET-EN) on bone mass; however, published studies on norethisterone (NET) have shown a positive effect on BMD (Horowitz et al 1993; Eldred et al, 1992; Riis et al, 1990). Combined oral contraceptives (COCs) have not been found to be associated with loss of bone mass (De Cherney, 1996; Mehta, 1993), with some studies indicating that COCs are bone-sparing (Mehta, 1993; Kleerkooper et al, 1991).

Few studies have investigated the effect of hormonal contraceptives on BMD in premenopausal long-term users of hormonal methods in their 40s, and none have investigated the effect of NET-EN on BMD.

DMPA and NET-EN are effective contraceptives with low failure rates (WHO, 1983). In South Africa, hormonal injectable contraceptive use is high, with about 60% of Black African women practising contraception using either DMPA or NET-EN (SADHS, 1998). Of the two available injections, older women mainly use DMPA and often continue to use this method into their late 40s. Use of COCs is lower with the method predominantly used by white and Asian women. As menopause is known to be associated with bone loss (Ahlborg et al, 2001), it would be important to know if hormonal methods of contraception could further contribute to bone loss in older women.

The aim of this study was to determine if long term use of hormonal contraceptives (COCs, DMPA and NET-EN) is associated with change in bone mass in women aged 40-49 years.

Subjects and Methods

A cohort of women aged 40 to 49 years old using DMPA, NET-EN, or COCs, and nonusers of hormonal contraception were recruited from a large family planning clinic in Durban, South Africa. Recruitment commenced in 2000 and was completed in 2003. For inclusion as a hormonal contraceptive user, women had to have used either DMPA, NET-EN or COCs for at least one year. For inclusion in the nonuser control group

women should not have used any form of hormonal contraception in the past year. All DMPA users were on a regimen of 150mg every 12 weeks and NET-EN users on 200mg every 8 weeks. The COCs used by women included a range of formulations, with most (93%) using formulations containing between 30-40mcg of estrogen. Women were eligible to participate if they had not lactated or delivered in the past 6 months, were not currently or had never used medication known to affect calcium metabolism for more than 3 months and did not have a chronic disease affecting calcium metabolism. Women who were postmenopausal were excluded from the study at screening as the aim of the study was to follow-up women through their natural menopause. Menopause was classified as amenorrhoea for at least one year. For injectable hormonal contraceptive users this criterion could not always be used as women commonly experienced amenorrhoea induced by the method. Therefore, in addition to amenorrhea, follicle-stimulating hormone (FSH) levels from blood samples were measured. An FSH level of ≥ 25.8 milli International Units per milliliter (mIU/mL) was considered to be in the menopausal range (King Edward VIII Hospital Durban; Chemical Pathology Laboratory criteria using Roche Elecsys FSH expected values).

On recruitment, a questionnaire was administered by a trained research nurse to elicit information on lifetime contraceptive history, fertility history, menopausal symptoms and regularity of the menstrual cycle. Questions were also asked on smoking, diet, exercise and caffeine and alcohol intake. The examination included height, weight, blood pressure and waist and hip measurements using a standard protocol. Body mass index (BMI) was calculated from the weight and height as kg/m^2 . Forearm BMD was measured

by dual energy x-ray absorptiometry (DXA model DTX-200), Osteometer MediTech A/S Co, Rodovre, Denmark). BMD was measured in grams/centimetre² (g/cm²) at two sites in the distal forearm (radius and ulna). The distal site contains predominantly trabecular bone and the cortical bone predominates in the ulna. The DXA equipment was standardized daily using a phantom as prescribed by the manufacturers instructions. Accuracy to the standard during the recruitment period was 0.53%. and in vivo precision was 0.94%. Study participants are currently being followed-up at six-monthly intervals for a total of five years. Women will be completing their five-year follow-up visit between 2005 and 2006. The findings presented in this paper are based on the baseline data collected at the recruitment visit and are cross-sectional in nature.

The characteristics of women in the study were quantified as means $\pm$ SD, medians, or percentages. Follicle-stimulating hormone levels were divided into two categories according to laboratory cut-off levels for premenopausal (<25.8 mIU/mL) and perimenopausal/menopausal ($\geq$ 25.8 mIU/mL). Differences in BMD between contraceptive groups, and the associations between BMD and selected characteristics of the study participants by contraceptive group were assessed using one-way analysis of variance and multiple variable linear regression for BMD measured at the two anatomic sites. The proposed study aims to be able to detect a half standard deviation difference in bone mass between users and nonusers of injectable contraceptives. This would be of biological significance as this difference would translate into a large difference in the risk of fracture in the older woman. Information on the mean and standard deviation of cross-sectional measurements of forearm bone mass in white, European, premenopausal

women reported by Nordin (Nordin, 1987) was used to estimate sample size. The sample size calculated assuming a two-tailed statistical test with a significance level of 5% and with power of 80% was 63 subjects in each contraceptive group. Loss to follow-up was estimated at approximately 10-15% per year. Therefore, sample size after adjusting for loss to follow-up was at least 100 women in each category.

Data were analysed using the statistical package STATA (V.8 College station, TX, USA). Ethical approval was granted by the University of the Witwatersrand, Human Subjects Research Committee, and by the Scientific and Ethical Review Group of the World Health Organization.

Results

In total, 496 women were recruited. Baseline information about these women is summarised in Table 3.6.1. Although the groups using hormonal contraception were similar in age, the nonusers of hormonal contraception were on average 2 years older than DMPA users ($p < .001$). Norethisterone enanthate users were the youngest group with a mean age of 43 years. Almost all women were African except for the COC group which included around a quarter (26%) of Indian women. This resulted in a significant difference in ethnicity between the groups ($p = .007$).

Table 3.6.1 Socio-demographic and lifestyle characteristics of subjects in the 40-49 year age range by contraceptive method use.

Characteristics	DMPA (n=127)	NET-EN (n=102)	COC (n=106)	Non user controls (n=161)	P value
Mean age, years (SD)	43.6 (2.7)	43.0 (2.2)	43.7 (2.5)	45.4 (2.5)	<0.0001
Mean highest Education Grade (SD)	8.5 (3.3)	9.8 (3.1)	9.8 (2.4)	9.1 (2.7)	0.012
Marital status %					<0.0001
Married/cohabiting	46.1	52.1	57.1	41.0	
Reg partner-not cohabiting	3.2	7.7	5.7	3.7	
Casual partner	38.9	36.5	28.6	25.5	
No partner	11.9	2.9	7.6	29.8	
Employment status %					<0.0001
Employed full/part-time	44.5	73.5	71.5	63.4	
Unemployed	54	19.6	23.8	33.5	
Housewife	1.6	6.9	4.8	3.1	
Ethnicity %					0.007
African	98.4	95.2	67.0	94.4	
Coloured	1.6	1.0	7.5	2.5	
Indian	0	3.8	25.5	3.1	
Exercise					0.48
No regular exercise (%)	96.9	96.0	94.3	93	
At least once a week (%)	3.1	4.0	5.7	7	
Dieted in last 6 months (%)	0	0	4.7	<1	0.003
Caffeine consumption*					0.08
Never (%)	9	18	19	12	
1-2 cups/day (%)	75	62	69	75	
> 2 cups (%)	15	19	12	12	
Ever smoked cigarettes (%)	11.0	10.3	10.4	16.1	0.17
Current smoker (%)	4.7	5.6	5.7	9.9	0.23

*Tea or coffee

DMPA= depot medroxyprogesterone acetate; NET-EN = Norethisterone enanthate;

COC= combined oral contraceptive; SD= standard deviation;

P value indicates differences between all groups

The reproductive characteristics of the woman are shown in Table 3.6.2. A number of other differences were noted between the groups including a significant difference in age of menarche, FSH, parity, employment, marital status and education. There were no differences in smoking history, exercise (Table 3.6.1) or years of lactation (Table 3.6.2).

Table 3.6.2 Reproductive characteristics by contraceptive method user group

Characteristics	DMPA (n=127)	NET-EN (n=104)	COC (n=106)	Nonuser (n=161)	P value
Parity (median)	4	3	3	3	0.04
Ever lactated %	88.2	91.3	87.7	83.2	0.34
Mean age at menarche, years(SD)	15.2±1.7	15.5±1.7	14.8±1.7	14.8±1.6	0.014
Lactation (yrs) median	3.5	3.2	3.0	3.2	0.06
FSH					<0.0001
< 25.8 mIU/mL, %	72	94	66	90	
≥ 25.8 mIU/mL, %	28	6	34	10	
Use of group method*					
Median use in last 5 yrs (months)	53	45	52	NA	
Median lifetime use (months)	84	49	89	NA	
Mean age at first use (years)	36	37	36	NA	

* Only group method shown i.e. group to which women using that method at the time of recruitment were allocated. Contraceptive history of nonuser group not shown.

All women using hormonal methods had used the method for at least 1 year at recruitment. The average length of lifetime use for DMPA was 9.3 years with a number of women (n=13) having used the method for 15 years or more (Table 3.6.2). Norethisterone enanthate users had been using the method on average for 5.6 years and COC users for 8.6 years.

There was no significant difference in BMD between the four contraceptive user groups at the radius (p=.26) or the ulna site (p=.21), with and without adjustment for age (Table 3.6.3). Although a small decrease in BMD was noted over the age range (40 to 49 years)

this was not statistically significant ($p=.7$). Length of use of method in the last 5 years and total lifetime use was not associated with difference in BMD. Although differences were noted between the contraceptive groups, most were not associated with BMD except for BMI, ethnic group and FSH level. A statistically significant difference in BMD was found between the Indian and African women ($p=.014$); however after adjusting for BMI the difference in BMD was no longer significant.

Bone mineral density was found to be associated with BMI ($p<.0001$) and with FSH level at the radius ($p=.029$) and ulna ($p=.022$). An increase of one unit of BMI was associated with an increase of 0.0044 g/cm^2 in radius and 0.0041 g/cm^2 in ulna BMD. Follicle-stimulating hormone level $\geq 25.8 \text{ mIU/mL}$ was associated with a decrease of 0.017 g/cm^2 in radius BMD (Table 3.6.3) relative to women with FSH $<25.8 \text{ mIU/mL}$.

Table 3.6.3 Factors potentially associated with Radius BMD (unadjusted)

Factors	Radius BMD g/cm^2 95% CI	P value
Contraceptive group		0.26
DMPA	0.514 (0.501-0.527)	
NET-EN	0.514 (0.499-0.528)	
COC	0.500 (0.486-0.514)	
Nonuser	0.518 (0.506-0.529)	
Ethnicity		
African	0.516 (0.509-0.523)	
Indian	0.491 (0.451-0.531)	0.007
Coloured	0.479 (0.456-0.503)	
Age, per year	-0.0017 (-0.0041-0.0008)	0.188
BMI, change per kg/m^2	0.0044 (0.0036-0.0052)	<0.001
FSH		0.029
$< 25.8 \text{ mIU/mL}$, %	0.516 (0.508)-0.524)	
$\geq 25.8 \text{ mIU/mL}$, %	0.498 (0.483-0.512)	

Body mass index, FSH level (equal to and above 25.8 mIU/mL versus below 25.8 mIU/mL) and interaction of BMD with FSH level were all significantly associated with BMD in a multiple regression model ($r^2 = 0.2$). According to this model, for women of median BMI of 33.9 units, mean radius BMD varied from 0.514 g/cm² [95% CI 0.507-0.521] with FSH < 25.8 mIU/mL, to 0.501 g/cm² [95% CI 0.488-0.513] for women with FSH $\geq$ 25.8 mIU/mL, (p=0.066). The effect of FSH on BMD was significantly modified by BMI, and vice-versa (p=.006). For women with FSH < 25.8 mIU/mL, BMD increased by 0.0038 [95%CI 0.0028-0.0047] (g/cm²) per unit increase in BMI, whereas for women with FSH $\geq$ 25.8 mIU/mL, BMD increased by 0.0067 g/cm² [95% CI 0.0048-0.0086] for each unit increase in BMI (Table 3.6.4). Similar results were obtained for ulna BMD (data not shown).

Table 3.6.4 Results of multiple regression model with BMI and FSH level

Factors	Radius BMD units 95% CI	P value
Effect of *FSH (for women of median BMI= 33.9 kg/m ²) < 25.8 mIU/mL $\geq$ 25.8 mIU/mL	0.514 (0.507-0.521) 0.501 (0.488-0.513)	<0.066
Effect of BMI, unit BMD per unit change in BMI For FSH< 25.8 mIU/mL For FSH $\geq$ 25.8 mIU/mL	0.0038(0.0028-0.0047) 0.0067 (0.0048-0.0086)	<0.001

* Significant interaction between FSH level and BMI (p=0.006)

Follicle-stimulating hormone level was found to be significantly different between user groups ($p < 0.0001$). However, after adjusting for age, the difference between the 4 groups was no longer significant ($p = 0.13$). An increase of one year in age increased FSH level by 3 mIU/mL ($p < 0.001$).

Discussion

The findings of this cross-sectional study of older woman show no evidence of a significant difference in BMD between the three contraceptive users and nonuser control groups. This is in agreement with some studies that have found no difference in BMD in long-term users of DMPA compared to nonuser controls (Petitti et al, 2000; Taneepanichskul et al, 1997) and the normal population mean (Gbolade et al, 1998). Some of these studies (Petitti et al, 2000; Gbolade et al, 1998) suggest that any bone loss would occur in approximately the first three years of use but that this effect could be reversible. However, other studies have shown that loss of BMD persists in long term users (Cundy et al, 2002; Pavia et al, 1999). The mechanism involved in reversing a hormonally initiated loss of BMD is not clear. It has been suggested (Petitti et al, 2000) that a homeostatic mechanism operates that reverses bone loss after prolonged hormonal stimulation. Women using contraceptives in the study reported here were mainly long term users and it may be that any initial loss of BMD associated with use of DMPA had been recovered.

While this study did not show that COC use was beneficial with respect to bone mass, some studies have shown a positive association of BMD with COCs (Taneepanichskul et

al, 1997; Kleerkoper et al, 1991). It could be speculated that a short-term increase in BMD as a result of COC use may return to the BMD levels of nonusers after some time. In support of this several studies have found that there is no difference in BMD in long-term users of COCs (Perrotti et al, 2001; Petitti et al, 2000).

This is the first study to include data on the 2-monthly injectable NET-EN. It is suggested that norethisterone (NET) may protect against calcium loss via a direct topic effect on bone or by the action of oestrogenic metabolites (Christiansen et al, 1985). Norethisterone enanthate can be considered to be similar in action to NET, as the enanthate (EN) is added primarily to give it its depot characteristics. Although the studies that have included NET (Horowitz et al, 1993; Eldred et al, 1992; Riis et al, 1990) have shown a positive effect on BMD, no positive effect of NET-EN was seen compared to other groups in this study.

Published studies have followed up women on treatment with NET for a year or less and therefore there are no data demonstrating that the positive effect of NET would be sustained in long term use. The lack of data on the effect of NET-EN on BMD may be because the method is not as widely used as DMPA. However in South Africa this method has increased in popularity in recent years and is often the method of choice for younger women (Smit et al, 2001).

No previous studies have reported on the relationship between FSH level and BMD which may be more important than age, as raised FSH is an indicator for menopause. In

our study, women with an FSH level of 25.8 mIU/mL or above had a significantly lower BMD at both distal sites. We have found a significant interaction between BMI and FSH in their effect on BMD. As a result of this, the loss in BMD for an increase in FSH level above 25.8 mIU/mL will be greater for women of lower BMI than for their counterparts with relatively higher BMI. For example, a woman with BMI at the 25th percentile of BMI in our sample (28.9 units) will lose 0.0285 g/cm² [95% CI-0.0462 to -0.0108] of BMD when moving from the lower category of FSH. By contrast, a woman at the 75th percentile of BMI (38.54 units) will lose only 0.000312 g/cm² [95% CI-0.018 to 0.0175] of BMD when her FSH level increases to more than 25.8 mIU/mL according to this model. In other words loss in BMD as a result of menopause is diminished by the effect of BMI.

In women with FSH $\geq$ 25.8 IU/mL BMD increased by 0.0067 g/cm² per unit increase of BMI whereas in women with FSH levels below 25.8 mIU/mL, BMD increased by 0.0038 g/cm² per unit increase in BMI. This means that the effect of BMI on bone density is greater for women with raised FSH levels, compared to women with FSH < 25.8 mIU/mL. Using the cut-off of FSH of 25.8 mIU/mL as a marker of menopause, the protective effect of BMI is greater for postmenopausal women (FSH $\geq$ 25.8 mIU/mL) than it is for pre-menopausal women (FSH <25.8 mIU/mL).

Since it has been suggested that there are differential effects of progestogen on cortical and trabecular bone (Gallager et al, 1991), with the protective effect being greater on cortical bone, differences in measurement sites and their composition may explain some

of the differences in effect of DMPA. In one study that found loss of BMD persisted in long-term use (Cundy et al , 2002), significant bone loss was found in only two of the four sites measured (trochanter and lumbar spine). No significant loss was found at the femoral neck and Ward's triangle sites. Similarly, another study (Gbolade et al, 1998) found there was a significant effect at the lumbar spine but no effect at the femoral neck. The distal forearm contains a greater proportion of cortical bone and some studies that have not found a difference in BMD with long term use have only used the forearm as a site of measurement (Petitti, et al 2000; Taneepanichskul et al, 1997). The issue of measurement site may be difficult to resolve as larger epidemiological studies may choose to take advantage of equipment that measures forearm BMD, which is less costly to purchase and maintain. This sort of equipment is not usually available in a clinical diagnostic setting where there is preference for measuring the hip and spine.

Investigation of women in this age range was considered important as any loss of BMD during short term use of DMPA could be exacerbated by age. There is, however, some debate around the extent of age related bone loss (Ahlborg et al 2001; Sowers et al 1993). One review (Nilas 1991) documents few studies demonstrating bone loss related to age in premenopausal women in the forearm, however, 8 of the 17 studies reviewed found that there was loss in the spine or femur. There was a small nonsignificant difference in BMD with age noted in the population in our study. Since the age range of our participants was narrow (10 years) and the forearm used as the only site of measurement, a significant difference in BMD and age may not have been expected.

This study confirms the association of BMD with BMI (Petitti et al, 2000; Tang et al, 2000; Pavia et al, 1999). African women had a significantly higher BMI and BMD compared to the Indian women in our study. Ethnicity itself has been reported to affect BMD with premenopausal black women having a greater BMD compared to white women (Liel et al, 1988). In the same study, further analysis restricted to both black and white non-obese women found that BMD was still higher in black women. Pettiti et al, (2000) found that the Black African women in Harare had a higher mean BMI and that ethnic differences in BMD persisted even after adjustment for BMI. However in our study differences in BMI explained the ethnic differences in BMD. Differences in BMI by racial groups have been reported previously in South Africa (Department of Health, South Africa, 2006).

The World Health Organization has developed a classification system which uses the population mean as a reference against which a BMD measurement can be compared to obtain a T-score. The T-score is expressed in the number of standard deviations (SDs) of difference from the normal population mean. The definition of normal BMD is regarded as no more than 1 SD below the young adult normal value. Osteopenia (low bone mass) is defined as 1 to 2.5 SDs below the population mean and below this level osteoporosis is diagnosed. The reference population used for the DXA equipment in this study included 20,000 Danish women with a mean distal (radius and ulna combined) BMD of 0.500 g/cm². There were no available population data in South Africa to compare this study group against. Using the data from this study the mean distal BMD (0.508 g/cm²) in the African population was slightly higher than the reference group. Both the coloured

(n=13) and Indian groups (n=38) had a lower distal BMD compared to the reference group (0.482 and 0.463 g/cm² respectively). However, the numbers in these two groups is low and may not be a good representation of these ethnic groups. Although a significant effect of DMPA on BMD has been reported elsewhere (Petitti et al, 2000 and Gbolade et al, 1998) this may not necessarily translate into concern of clinical importance so long as the deficit is still within the 1 SD of the normal value.

Although some studies have reported that bone loss is associated with lactation (Lamke et al, 1976), there appears to be recovery in the long term (Lamke et al, 1976). Length of lactation and its association with BMD is also unresolved (Sowers et al, 1993). Our study found no association between length of lactation and BMD.

In conclusion, long term use of hormonal methods in this study population does not negatively impact on BMD and there should not be concerns about allowing women to continue using this method until they reach menopause. Further, African women in our study had higher BMIs than other population groups however after adjusting for BMI there was no difference between the population groups in this age groups.

Limitations of the study

The cross-sectional nature of the study is a limitation. The women recruited are now contributing to a longitudinal study with five years follow-up. BMD measurements were limited to the forearm and therefore findings may not be applicable to other skeletal sites.

3.7 Bone mineral density in older long-term users of depot-medroxyprogesterone acetate, norethisterone enanthate or combined oral contraceptives for Contraception: A longitudinal study

Mags E Beksinska¹, Immo Kleinschmidt², Jenni A Smit¹, Timothy MM Farley³.

This paper is being prepared for submission

¹Reproductive Health and HIV Research Unit, Department of Obstetrics and Gynaecology, University of the Witwatersrand, Mayville, 4091, South Africa

²London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E

³UNDP/UNFPA/WHO/World Bank Special Programme of Research, Development and Research Training in Human Reproduction, CH 1211 Geneva 27, Switzerland.

Introduction

Most studies have found that current users of depot-medroxyprogesterone acetate (DMPA) have lower bone mineral density (BMD) compared to nonusers (Curtis & Martins, 2006, Wanichsetakul et al, 2002; Petitti et al, 2000; Scholes et al, 1999; Cundy et al, 1996; Cundy et al, 1991). There is limited information in women over 40 years of age (Sanches et al, 2008, Beksinska et al, 2005, Tang et al, 2000, Tang et al, 1999, Gbolade et al, 1998) as few studies have included women in this age group. Results of these studies have been mixed with some finding no differences in BMD between older DMPA users and nonusers or normal population means (Sanches et al, 2008, Beksinska et al, 2005, Gbolade et al, 1998) and those that have found a negative effect of DMPA on BMD compared to nonusers (Rosenburg et al, 2007, Tang et al, 2000, Tang et al, 1999)

Studies investigating COC use in perimenopausal users have not found any negative impact on BMD compared to nonusers of COCs (Martins et al, 2006). Two cross-sectional studies have looked at BMD in norethisterone enanthate users (NET-EN) (Rosenburg et al, 2007, Beksinska et al, 2005). In one of these studies (Rosenburg et al, 2007) current NET-EN users aged 40-44 had lower ultrasound measures in the calcaneus compared to nonusers, while the second study found no difference in forearm BMD between current users and controls (Beksinska et al, 2005). This study aimed to investigate BMD in older users (40 to 49 years) of DMPA, NET-EN, COC and nonusers of contraception in a 4-5 year follow-up study.

Subjects and Methods

A cohort of women aged 40 to 49 years old using DMPA, NET-EN, or COCs, and nonusers of hormonal contraception were recruited from a large family planning clinic in Durban, South Africa. Recruitment commenced in 2000 and was completed in 2003. Details of the study methodology are described in chapter 3.6 and previously published (Beksinska, 2005). Briefly, for inclusion as a hormonal contraceptive user, women had to have used either DMPA, NET-EN or COCs for at least one year. For inclusion in the nonuser control group women should not have used any form of hormonal contraception in the past year. Women who were postmenopausal were excluded from the study at screening using menstrual history and follicle-stimulating hormone (FSH) levels from blood samples. An FSH level of ≥ 25.8 milli International Units per milliliter (mIU/mL) was considered to be in the menopausal range (King Edward VIII Hospital Durban; Chemical Pathology Laboratory criteria using Roche Elecsys FSH expected values).

On recruitment, a questionnaire was administered to elicit information on lifetime contraceptive history, fertility history, menopausal symptoms and regularity of the menstrual cycle. The examination included height, weight, blood pressure and waist and hip measurements using a standard protocol. Forearm BMD was measured by dual energy x-ray absorptionmetry (DXA model DTX-200). Osteometer MediTech A/S Co, Rodovre, Denmark). BMD was measured in grams/centimetre² (g/cm²) at two sites in the distal forearm (radius and ulna). The DXA equipment was standardized daily using a phantom as prescribed by the manufacturers instructions. Accuracy to the standard during the recruitment period was 0.53%. and in vivo precision was 0.94%. Study

participants were followed-up at six-monthly intervals for a total of four to five years depending on time of recruitment. Women completed their follow-up visit between August 2005 and April 2006.

The characteristics of women in the study were quantified as means $\pm$ SD, medians, or percentages. Follicle-stimulating hormone levels were divided into two categories according to laboratory cut-off levels for premenopausal (<25.8 mIU/mL) and perimenopausal/menopausal (≥ 25.8 mIU/mL). Differences in BMD between contraceptive groups, and the associations between BMD and selected characteristics of the study participants by contraceptive group were assessed using one-way analysis of variance and multiple variable linear regression. The sample size calculated assuming a two-tailed statistical test with a significance level of 5% and with power of 80% was 63 subjects in each contraceptive group. Sample size after adjusting for potential loss to follow-up was at least 100 women in each category. Data were analysed using the statistical package STATA (V.10 College station, TX, USA). Ethical approval was granted by the University of the Witwatersrand, Human Subjects Research Committee, and by the Scientific and Ethical Review Group of the World Health Organization.

Results

In total, 496 women were recruited. Baseline information about these women is summarised in Table 3.7.1. Although the groups using hormonal contraception were similar in age (43 years), the nonusers of hormonal contraception were on average 2 years older than DMPA users ($p<.001$). Almost all women were African except for the

COC group which included around a quarter (26%) of Indian women. Most women had started using the group method in their mid-thirties on completion of desired family size and had used for approximately 4 years on recruitment. Few women were current smokers.

Table 3.7.1 Baseline characteristics of subjects in the 40-49 year age range by contraceptive method use

Characteristics	DMPA (n=127)	NET-EN (n=102)	COC (n=106)	Non user controls (n=161)	P value
Mean age, years (SD)	43.6 (2.7)	43.0 (2.2)	43.7 (2.5)	45.4 (2.5)	<0.001
Ethnicity %					0.007
African	98.4	95.2	67.0	94.4	
Coloured	1.6	1.0	7.5	2.5	
Indian	0	3.8	25.5	3.1	
Exercise					
No regular exercise (%)	96.9	96.0	94.3	93	0.48
At least once a week (%)	3.1	4.0	5.7	7	
Dieted in last 6 months (%)	0	0	4.7	<1	0.003
Current smoker (%)	4.7	5.6	5.7	9.9	0.23
Parity (median)	4	3	3	3	0.04
Ever lactated %	88.2	91.3	87.7	83.2	0.34
Mean age at menarche, years(SD)	15.2± 1.7	15.5±1.7	14.8±1.7	14.8±1.6	0.014
Lactation (yrs) median	3.5	3.2	3.0	3.2	0.06
FSH					
< 25.8 mIU/mL, %	72	94	90	68	<0.0001
≥ 25.8 mIU/mL, %	28	6	10	32	
Use of group method		45	52	NA	
Median use in last 5 yrs (months)	53	49	89	NA	
Median lifetime use (months)	84	37	36	NA	
Radius BMD g/cm ²	0.514	0.514	0.500	0.518	0.26

* Only group method shown i.e. group to which women using that method at the time of recruitment were allocated. Contraceptive history of nonuser group not shown.

DMPA= depott medroxyprogesterone acetate; NET-EN = Norethisterone enanthate;
COC= combined oral contraceptive; SD= standard deviation;

There was no significant difference in BMD at baseline between the four contraceptive user groups at the radius ($p=.26$) or the ulna site ($p=.21$), with and without adjustment for age. Although a small decrease in BMD was noted over the age range (40 to 49 years) this was not statistically significant ($p=.7$). Length of use of method in the last 5 years and total lifetime use was not associated with difference in BMD.

Although differences were noted between the contraceptive groups, most were not associated with BMD except for BMI, ethnic group and FSH level. A statistically significant difference in BMD was found between the Indian and African women ($p=.014$); however after adjusting for BMI the difference in BMD was no longer significant.

During follow-up all nonusers of hormonal contraception remained as nonusers, however many women in the user groups continued participation in the study but ceased using a contraceptive method. At baseline 32% of women recruited were nonusers of contraception, this increased to 71% after three years of follow-up and by end of the follow-up period the majority of women (87%) had ceased using a method of contraception. Due to small numbers of hormonal contraceptive users from 3 years of follow-up, comparison of user groups was conducted at the 2.5 year visit. At this follow-up visit 278 women continued with the same method they were using at baseline. Women were excluded from the analysis if they stopped or changed their method. No difference was found in BMD between the groups at this interim analysis (Table 3.7.2).

Table 3.7.2 Mean Radius BMD at 2.5 years by contraceptive group*

	N	Radius BMD g/cm ² (SD)	P value
Contraceptive group			0.522
DMPA	63	0.511 (.071)	
NET-EN	38	0.501 (.081)	
COC	48	0.500 (.082)	
Nonuser	129	0.504 (.075)	

*Only women continuing with the same method from baseline were included in this analysis

Further analysis is underway to complete this paper

Discussion

This longitudinal study found no difference in forearm BMD between users and nonusers of hormonal contraception after 2.5 years of follow-up. This is in agreement with data collected at baseline in our study (Beksinska et al, 2005). The populations investigated in studies looking at the effect of DMPA and NET-EN have included women using the method in their 40s but there is limited information in this age group specifically. The age ranges of women in some studies have included users up to the age of 52, however the numbers have been small. In one cross-sectional study older DMPA users were disaggregated in the data (Gbolade et al, 1998) and no differences were found in BMD in women aged between 40-49 and a slightly older group of 50-52 compared to a normal population mean in the lumbar spine and femoral neck. The population of women in the Tang et al (1999) cross-sectional study were generally older than other studies with a mean age of 43 years. In this study those who had used DMPA for 5 years or more had significantly lower BMD in the spine, femoral neck, trochanter and ward's triangle compared to never users. From the initial cross-sectional sample of 67 women, 59 were

followed up in the 3-year prospective component of the study (Tang et al, 2000). Small losses were noted of less than 1% in all sites but not the trochanter. In addition there was no effect seen with duration of use aside from a weak negative between BMD in the femoral neck and duration of use. One longitudinal study (Sanches, 2008) followed up women who had been long-term users of either DMPA or the IUD until menopause. This study found no difference in forearm BMD between these two groups at each of the three sites at one-year follow-up post-menopause.

In summary there is limited data available on DMPA use in this group of older women due to the low prevalence of use in this age group and information in this age group has come from studies including a wider age range of women. However, loss of BMD has been found in these older DMPA users.

Two cross-sectional studies have looked at BMD in norethisterone enanthate users (NET-EN) (Rosenburg et al, 2007, Beksinska et al, 2005). In one of these studies (Rosenburg et al, 2007) a similar negative effect was found in current DMPA and NET-EN users aged 40-44 who had lower ultrasound measures in the calcaneus compared to nonusers, while the second study found no difference in forearm BMD between current NET-EN users and controls (Beksinska et al, 2005). Both cross-sectional and longitudinal studies investigating COC use in perimenopausal users have not found any negative impact on BMD compared to nonusers of COCs (Martins et al, 2006). Further analysis of the older woman in this dataset will consider menopausal status and also look at changes in BMD in women who ceased using hormonal contraception during follow-up.

3.8 Detection of raised FSH levels amongst older women using depot-medroxyprogesterone acetate and norethisterone enanthate

Mags E Beksinska, Jenni A Smit¹, Immo Kleinschmidt², Helen V. Rees¹,
Timothy MM Farley³, Franco Guidozi⁴

This paper has been published in *Contraception* 2003; vol.68, no.5, pp.339-43.

(Appendix 8)

¹The Reproductive Health Research Unit, Department of Obstetrics and Gynaecology, University of the Witwatersrand, Suite 1301, Maritime House, 143 Salmon Grove, Durban, South Africa

²Family Health International, P.O Box 13950, Research Triangle Park NC 27709, USA

³Special Programme for Research, Development and Research Training in Human Reproduction, Department of Reproductive Health and Research, World Health Organisation, Geneva, Switzerland.

Department of Obstetrics and Gynaecology, Johannesburg Hospital, University of the Witwatersrand, Johannesburg, South Africa

Abstract

Background: The objective of this study was to investigate whether follicle-stimulating hormone (FSH) levels can be used reliably to indicate approaching menopause in older (aged 40-49) long-term users of depot-medroxyprogesterone acetate (DMPA) and norethisterone enanthate (NET-EN).

Study design: One-hundred and seventeen women using DMPA, 60 NET-EN users and 161 nonusers of contraception were recruited. At recruitment, serum FSH levels were measured and questions were asked regarding menopausal symptoms, menstrual cycle and date of last injection.

Results: Results of the recruitment blood test showed that 32% of the nonusers had FSH levels in the menopausal range >25.8 milli International units per milliliter (>25.8 mIU/mL) compared to 28% of the DMPA users and 9% of the NET-EN group. After adjusting for age, there was no significant difference between the 3 groups ($p=0.13$). An increase of one year in age increased the FSH level by 3 mIU/mL ($p<0.001$). All the hormonal contraceptive users were between one day and 12 weeks of their injection interval. Many had been using the injectable contraceptive method for over 10 years and almost all were amenorrheic at the time of recruitment.

Conclusion: The data show that a raised FSH level can be detected during use of DMPA and NET-EN and could be used as a menopausal indicator without interrupting method use in this group of contraceptive users.

Introduction

Depot-medroxyprogesterone acetate (DMPA) and norethisterone enanthate (NET-EN) are effective contraceptives with low failure rates (WHO, 1983). One of the common side effects of these methods is amenorrhea, which increases in incidence over time (WHO, 1983). In older users it may be difficult to distinguish between amenorrhea associated with approaching menopause and that induced by the method. Detection of menopause or perimenopause, therefore, presents a challenge in this group of contraceptive users. Traditional textbook management of an older woman using oral hormonal contraception recommends measurement of follicle-stimulating hormone (FSH) on the seventh day of the pill-free interval (Guillebaud, 1993). Advice for DMPA and NET-EN users is less clear; however, in the absence of data documenting efficacy it has been suggested that they should change to a nonhormonal method of contraception, as menopausal symptoms will be masked, and measurement of FSH levels may be unreliable (Guillebaud, 1993).

DMPA and NET-EN are known to inhibit the mid-cycle surge of FSH and luteinising hormone (LH), but the tonic release of these gonadotrophins continues at luteal phase levels (Mishell, 1972; Franchimont, 1970). A few studies have also shown that DMPA and NET-EN suppresses raised FSH and LH levels in postmenopausal women (Perez-Palacois, 1981; Fotherby, 1977). While limited information exists on the duration of the suppression of raised FSH and LH levels associated with menopause, in both these studies (Perez-Palacois, 1981; Fotherby, 1977), the lowest FSH and LH levels occurred between 14-30 days after injection. The majority of suppressed levels were still high enough to be classified as in the menopausal range. Thereafter, FSH and LH levels started to rise, and in

one of these studies (Perez-Palacois; 1981), FSH levels started rising back to pre-suppression levels between 40-80 days after injection.

In South Africa, hormonal injectable contraceptive use is high, with about 60% of Black African women practicing contraception using either DMPA or NET-EN (Department of Health, South Africa, 1999). Of the two available injections, older women almost exclusively use DMPA and often continue to use this method into their late 40s.

While the primary aim of this study was to investigate bone density in older women using hormonal contraceptives, a secondary objective was to investigate whether FSH levels could be used as an indicator of menopausal status in injectable users. This was due to the high prevalence of amenorrhea in the two injectable groups which made it impossible to use the menstrual cycle as an indicator of menopausal status. This paper presents the results of the latter component of the study.

Materials and methods

A cohort of older women using DMPA (150 mg at 3-monthly intervals), NET-EN (200 mg at 2-month intervals), combined oral contraceptives (COCs) and nonusers of contraception were recruited from a large family planning clinic in Durban, South Africa, between September 2000 and November 2001. For inclusion as an injectable user, women had to be aged between 40 and 49 years and had to have used the injectable hormonal contraceptive for at least one year. On recruitment, a questionnaire was administered which elicited information on contraceptive history, menopausal symptoms and regularity of the

menstrual cycle. Since women were recruited at different times in the injection cycle, date of last injection was recorded. A sub-sample of injectable users with FSH levels >25.8 mIU/mL at recruitment were contacted and asked to return for a second test to confirm their earlier result. If the recruitment measurement was taken later in the injection cycle, the woman was asked to return for a repeat test in the expected peak suppression period in her next injection cycle (14-30 days). If the recruitment measurement was taken during time of expected peak suppression she was asked to return after this time in the next injection cycle. FSH measurements are repeated yearly for the 5-year duration of the study.

A serum sample was obtained at the recruitment visit, and serum FSH levels were determined by immunoassay on the Roche MODULAR ANALYTICS E170 (Elecsys module) immunoassay analyser. Repeated samples from each subject were always analyzed in the same FSH assay batch. In total, 8 assays were conducted during the time of data collection for this study. For the purposes of this study, an FSH level between 25.8 and 135 milli International Units per milliliter (mIU/mL) was considered to fall into the menopausal range (King Edward VIII Hospital Durban; Chemical Pathology Laboratory criteria using Roche Elecsys FSH expected values). *Assay specificity:* With respect to analytical specificity, the following cross reactions with other hormones have been determined for the monoclonal antibody used: leutenising hormone, thyroid stimulating hormone, human chorionic gonadotropin, human growth hormone and human placental lactogen at <0.1%. *Assay sensitivity:* The lower detection limit is <0.10 mIU/ml. The detection limit represents the lowest FSH level that can be distinguished from zero. It is

calculated as the concentration lying two standard deviations above that of the lowest standard. The maximum of the measuring range is 200 mIU/ml. Values above this figure are reported as >200 mIU/ml. All samples analyzed for this study were above the lower detection limit and no sample was reported over 200 mIU/ml. *Test precision* (reproducibility): The within-assay coefficients of variation ranged between 0.78 and 1.5% for QCs with mean values of 9.53 and 33. Between assay variability ranged from 3.5 to 7.6% depending on the serum FSH concentration.

To investigate whether DMPA and NET-EN interfered with the FSH assay, control test samples and patient samples with high and low values of FSH were spiked with both contraceptives at levels above the highest circulating levels of MPA and NET described in the literature (Mishell, 1996; Fotherby, 1980). The results of this showed that only very small differences were observed, which fell into the quoted within-run precision of the test.

In this article we present the data collected up to November 2001 relating to the injectable hormonal users. Data on COC users will be analyzed separately. Mean and median FSH levels were compared between nonusers and each of the 2 injectable user groups, by means of Student's t-test and the Wilcoxon test, respectively, using the statistical package STATA (V.7 College Station, TX, USA). Multiple regression was done to investigate the effect of hormonal injectable use on FSH, adjusted for age. Ethical approval was granted by the University of the Witwatersrand, Human Subjects Research Committee.

Results

In total, 117 DMPA and 60 NET-EN users were recruited. Fewer women were recruited in the NET-EN group, as this method is not as popular in older women. Baseline information about these women and 161 nonusers of contraception is summarized in Table 3.8.1. Nonusers of hormonal contraception were on average 2 years older than DMPA users ($p<0.001$). NET-EN users were the youngest group with a mean age of 42.5 years. All women using either DMPA or NET-EN had been using the method for at least one year at recruitment. The average length of lifetime use for DMPA was 9.3 years, with a number of women ($n=13$) who had used the method for 15 years or more.

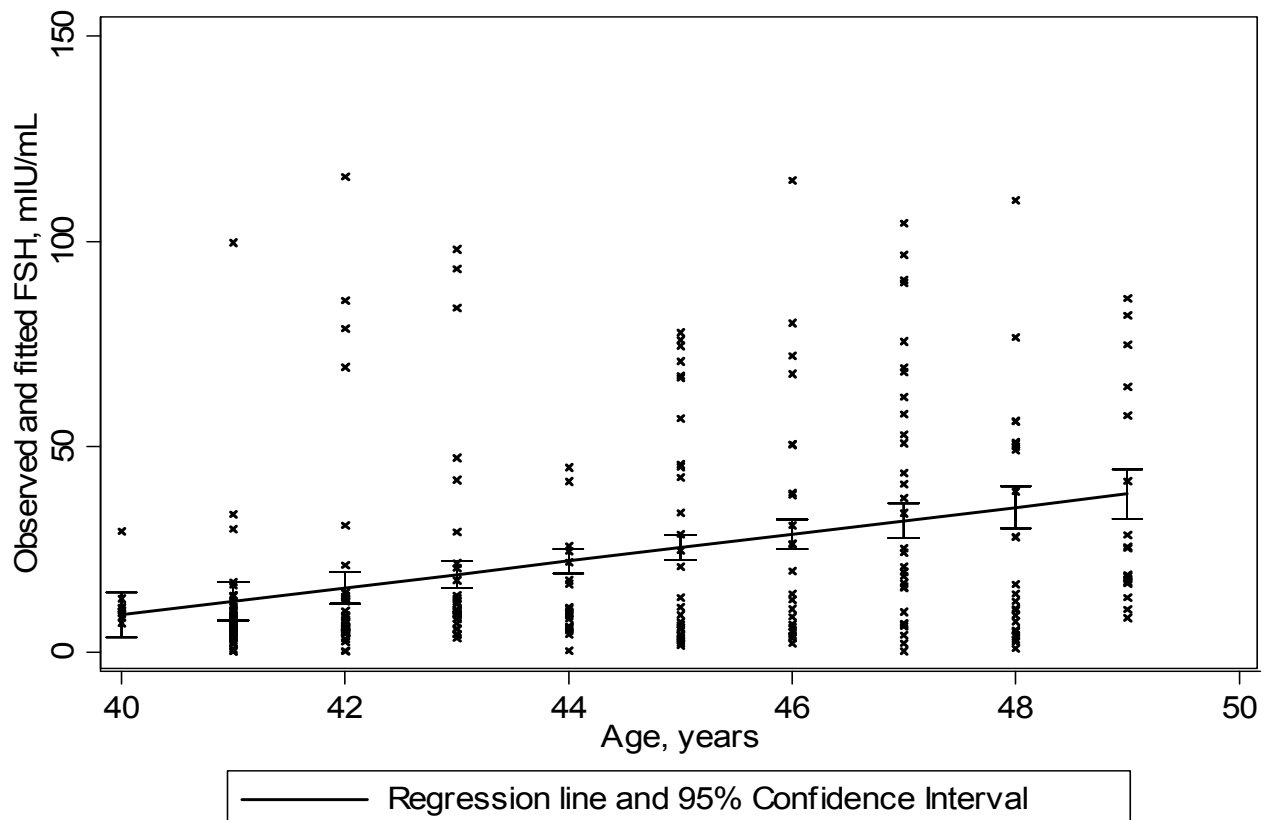
Table 3.8.1 Baseline information

	Nonusers N=161	DMPA Users N=117	NET-EN Users N=60
Mean Age (yrs) ($p<0.001$)	45.3	43.6	42.5
Median	46	43	42
Range	41-49	41-49	41-49
Mean FSH mIU/mL ($p=0.013$)	26.4	22.1	11.6
Median ($P=0.013$)	12.6	10.1	7.2
Range	0.2-115	0.1-114	0.2-51.1

NET-EN users had been using the method on average for 5.6 years. The majority of subjects had started using DMPA or NET-EN immediately after the birth of their last child, as they had attained their desired family size. Very few women using DMPA reported regular menstrual cycles (8%); women not reporting a regular cycle were in almost all cases experiencing spotting or amenorrhea. More women in the NET-EN group (48%) reported regular cycles. Results of the recruitment blood test showed that

32% of the nonusers had FSH levels in the menopausal range ($>25.8\text{mIU/mL}$) compared to 28% of the DMPA users and 9% of the NET-EN group. FSH was associated with a woman's age ($p=0.013$), and Figure 3.8.1 shows a plot of FSH levels by age predicted from the regression model. After adjusting for age, there was no significant difference in FSH levels among the 3 groups ($p=0.13$). An increase of one year in age increased the FSH levels by 3 mIU/mL ($p<0.001$).

Figure 3.8.1 Observed and fitted FSH levels by age.



Of the total 33 women using DMPA and 6 using NET-EN who had an FSH level greater than 25.8 mIU/mL on their recruitment visit, 12 DMPA and 2 NET-EN users were able to return during their next injection cycle after being contacted by telephone. Table 3.8.2 shows the 2 results in each of these participants. In total, 7 women had one of the 2 FSH measurements taken in the time of expected peak suppression (14-30 days). In 3 of these measurements the FSH level fell below the menopausal range (<25.8mIU/mL) while the other 4 still remained in the menopausal range

Table 3.8.1 Individual FSH levels of the sub-group of users of DMPA and NET-EN with raised FSH levels at recruitment

Subject number	Days post –injection earlier measurement	FSH (mIU/mL)	Days Post- injection later measurement	FSH (mIU/mL)
DMPA				
391	50	52.9	85	71.8
584	42	38.7	48	37.4
628	27	28.6	37	57.9
629	14	113	42	99.7
632	14	22.9	90	45.6
703	25	9.3	72	93.3
706	76	32.7	90	45.1
734	55	54.3	93	68.2
740	52	54.6	91	50.5
792	17	67.9	36	69.4
822	5	30.8	14	33.4
833	1	50.2	28	41.5
NET-EN				
639	15	21.3	38	28.5
724	10	27.4	60	51.2

(>25.8mIU/mL). The levels in most cases differed considerably depending on the time post-injection. The level taken later in the injection cycle was higher in 10 of the 14 cases. In 2 subjects (629,792), similar FSH levels were observed during expected peak suppression (14-30 days) and later in the injection cycle.

Discussion

Previous studies have been limited due to their small sample sizes and the inclusion of subjects who received only one dose of MPA or NET-EN (Perez-Palacois, 1981; Fotherby, 1977). These studies concluded that the baseline values of FSH and LH in postmenopausal women declined from the day of injection to reach their lowest levels between 14 and 30 days and then rose back to pre-suppression levels between about 40 and 80 days post-injection. In one of these studies (Fotherby, 1977), one of the subjects received a second dose of NET-EN and results indicated that the suppression of FSH was not as pronounced as that of the first dose. Further, the degree of suppression of FSH is reported to vary considerably between women, with suppression ranging between 30-75% of baseline values (Fotherby, 1977). Although a great deal of variability between women was shown, a similar pattern of suppression after injection and return to baseline values later in the dosing cycle was exhibited (Fotherby, 1977).

Our study supports the earlier work, in that in almost all cases, DMPA and NET-EN did suppress FSH levels in the first 30 days after injection. Where 2 samples were taken in the same subject, the levels of FSH measured in the sample taken sooner post-injection were lower in almost all cases. In 2 cases, the gonadotrophin levels were measured on day 1

(subject 833) and day 5 (subject 822) post-injection, probably before peak suppression occurred. In one case (833) the level taken subsequently at day 28 during the time of expected peak suppression was found to be lower, while in the other (822) the level was slightly higher. It may be that in this subject the FSH level was already suppressed by day 5 and by day 14 it was recovering.

In some subjects both measurements were taken after the time of expected peak suppression and in these cases the differences between the 2 measurements was smaller; however, the measurement taken later in the cycle was similar or higher. The subject with the highest FSH level (subject 629) showed little difference between her level at 13 days (113) and that taken at 42 days (99.7). It may be that the peak suppression of FSH occurred between these 2 times or that gonadotrophin levels in this subject only responded marginally in terms of suppression. This subject had been using the method for 14 years in total.

This is the first study which examines FSH levels in long-term users of DMPA and NET-EN. Raised FSH levels were found in women during use of the method and this indicates that measurement of gonadotrophins could be used as an indicator of onset of menopause. This also supports earlier work where gonadotrophin levels rarely fell below a peak level of 20 mIU/mL in postmenopausal women (Perez-Palacois, 1981; Fotherby, 1977), again suggesting that the suppression would not necessarily make gonadotrophin levels unreliable indicators of menopause in hormonal injection users. It does suggest, however,

that the measurement should be taken as late as possible in the 2- or 3-month injection cycle to ensure that the gonadotrophin levels were approaching baseline levels.

There are no local guidelines in South Africa on how to deal with a woman using injectable contraception in her later reproductive years; however, the policy at a large provincial hospital in Durban is to refer users reaching 50 years, or users who report menopausal symptoms, for FSH measurement at the end of a 3-month DMPA injection cycle (King Edward Hospital, Durban, personal communication). If an FSH level is found to be in the menopausal range, the woman will be counselled to consider changing her method of contraception to a barrier method to see if menses returns. Raised levels of FSH with accompanying menopausal symptoms have been documented at this facility amongst hormonal injection users. This finding has important implications for guiding family-planning providers in the management of older women using hormonal injectable contraception. Women could possibly have gonadotrophin levels measured without discontinuing the method, as long as the test is taken later in the injection cycle for both NET-EN and DMPA. A good time to measure levels is on return for the next injection dose before the gonadotrophin levels are reduced.

The transition to menopause described as the perimenopause has been estimated to be just under 4 years in some populations (McKinley, 1992). As a woman moves into the perimenopause, FSH can fluctuate between pre- and post-menopausal values and menopausal symptoms such as erratic menstrual cycles, and hot flushes will be experienced (Sherman, 1975). The variability of FSH levels during this time has called

into question the reliability of using the FSH measure to diagnose or predict menopause (Burger, 1994). However, in injectable users, it is the only means of assessing the menopause and, based on our findings, it should still be considered an option.

Further work will be done in a more in-depth study on this issue, which will involve taking multiple samples in a smaller subset of women who have agreed to participate in having repeat samples taken at short intervals within the dosing interval.

Limitations of the study

This study had a number of limitations. Subjects were part of another study which was not set up for taking multiple blood samples which would be required to obtain an in-depth picture of gonadotrophin suppression. The researchers aimed to get subjects to come once during the peak gonadotrophin suppression period and once towards the end of a 2- or 3-month injection cycle. This was not always possible as many women were only contactable by letter and it was therefore difficult to remind them to come at the exact time scheduled. The laboratory had not developed its own reference ranges for the FSH test.

4.0 CONCLUSION AND RECOMMENDATIONS

This conclusion briefly summarizes overarching issues from the discussion sections of the main papers and highlights the key evidence emerging from this thesis. This study has added to the existing body of evidence on the effect of hormonal contraception on BMD. It has provided new data on the effect of the injectable contraceptive NET-EN and it has investigated BMD in mixed hormonal contraceptive users. It is one of few studies on BMD undertaken amongst African users of hormonal contraceptives. Through publication and other modes of dissemination, the findings have been made available to the wider scientific community and policy makers at national and international level.

In summary, this study found that although radius BMD increased during follow-up in adolescents and young women in both non-user and hormonal contraceptive users, there was evidence of lower bone mass in NET-EN and COC groups compared to the non-user group. Although DMPA users had a lower yearly increase in forearm BMD compared to non-users, this was not significant. Other studies using the forearm as a measurement site have generally not found significant differences between DMPA users and controls (Bahamondes, et al, 1999, Tharnprisarn & Taneepanichskul, 1999).

At the end of the follow-period in this study the adolescent group were aged between 19 and 24 years old. A final measurement of BMD in the hip, spine and femoral neck was taken in a sub-sample of 96 women in group which found evidence of lower BMD values in injectable users (including mixed injectable users) compared to non-users of hormonal contraception, but not among those women who had used COCs in combination with

injectables. This is in agreement with other longitudinal studies of BMD in adolescents using DMPA alone (Cromer et al, 1996, Cromer et al, 2004, Larra-Torre, 2004).

The longitudinal component of the study has added evidence that use of COCs in adolescents may result in lower bone growth. In the cross-sectional component of the study, there were not enough women continuing use of COCs exclusively to comment on BMD change. The group that had mixed COC users with injectable use over a 4-5 year period had lower BMD values but this was not significantly different from the non-users and the sample was small.

Our study shows some evidence of a recovery in growth in BMD in a very small sample of NET-EN discontinuers who did not use another hormonal method of contraception after cessation. Although no other longitudinal data exists on women who have discontinued use of NET-EN, this is in agreement with the only cross-sectional study that investigated women who commenced NET-EN during adolescence and then ceased using the method (Rosenburg, et al).

In the older users aged 40-49 years there were no differences found in BMD between users of DMPA, NET-EN and COCs and non-users of hormonal contraception at baseline. Many women had been using hormonal methods for more than 10 years at recruitment. No difference was noted in BMD between the groups in an interim analysis at 2.5 years. In this age group BMD was found to be associated with FSH level and raised FSH levels were detected in users of hormonal contraception. As mentioned above

studies measuring the forearm have been less likely to find an effect of hormonal contraception on BMD. Due to cost issues it was not possible to include older women in the final cross-sectional measurement of the central sites.

Several regulatory bodies have cautioned against long-term use of DMPA (FDA, 2004, MHRA, 2004, WHO, 2006). In the absence of evidence on NET-EN the WHO applied the same criteria for NET-EN use (WHO, 2006). No restriction has been placed on use of other progestogen-only or combined hormonal contraceptive methods. There are now concerns that these recommendations need to be reviewed in the light of emerging evidence of BMD reductions in low-dose COC users (Alberatzzi et al, 2006, Berenson et al, 2008). In addition as evidence grows on recovery of BMD post discontinuation of DMPA and NET-EN there is a need to balance loss of BMD against the benefit of using effective contraception, especially in adolescents.

Recommendations for future research

The issue of recovery of BMD in ex-users of DMPA and NET-EN requires longitudinal studies, with larger sample sizes and longer periods of follow-up. Although data is showing adolescents recover BMD on cessation of use of DMPA and this study has shown recovery in adolescents discontinuing NET-EN, follow-up is needed beyond acquisition of peak bone mass to determine if full recovery of BMD is made in women who use DMPA, NET-EN and low dose COCs as adolescents. A study to address longer term follow-up is currently in the planning stage and will commence in 2010.

The mechanism by which DMPA can reduce BMD is well documented (Ortiz et al, 1977), however there is less information on NET-EN and the mechanisms by which it could affect BMD and this needs to be investigated further.

APPENDIX 1

CONSENT FORMS AND INFORMATION SHEETS (ENGLISH AND ZULU)

SUBJECT INFORMATION SHEETS: ENGLISH

Aged 15 – 19 users of hormonal contraceptives

Aged 15 -19 non-users of hormonal contraceptives

Aged 40 – 49 users of hormonal contraceptives

Aged 40 -49 non-users of hormonal contraceptives

SUBJECT INFORMATION SHEETS: ZULU

Aged 15 – 19 users of hormonal contraceptives

Aged 15 -19 non-users of hormonal contraceptives

Aged 40 – 49 users of hormonal contraceptives

Aged 40 -49 non-users of hormonal contraceptives

CONSENT FORMS: ENGLISH

Aged 15 – 19 users of hormonal contraceptives

Aged 15 -19 non-users of hormonal contraceptives

Aged 40 – 49 users of hormonal contraceptives

Aged 40 -49 non-users of hormonal contraceptives

CONSENT FORMS: ZULU

Aged 15 – 19 users of hormonal contraceptives

Aged 15 -19 non-users of hormonal contraceptives

Aged 40 – 49 users of hormonal contraceptives

Aged 40 -49 non-users of hormonal contraceptives

CONSENT FORMS: ENGLISH & ZULU: SUBSTUDY OF SPINE/HIP/NECK DEXA

Information sheet, Consent form English and Zulu

SUBJECT INFORMATION SHEET
(15-19 age group, users of hormonal contraceptives)

INJECTABLE AND ORAL CONTRACEPTIVE BONE MASS STUDY

What is this study about?

The injectable contraceptives Depo Provera and Nuristerate and oral contraceptives (pills) have been used by women in this country for many years. The Reproductive Health Research Unit based in the Department of Obstetrics and Gynaecology of Baragwanath Hospital is undertaking a study to look at the effect of these contraceptives on the bones of the skeleton (bone mass).

What will be involved If I take part in this study?

We are looking for women volunteers aged between 15 and 19 who want to use the injectable Depo-Provera, Nuristerate or oral contraceptives to take part in this study. If you decide to volunteer, A nurse will interview you to collect information on your medical history, contraceptive history, and other reproductive information including pregnancies and breastfeeding. The nurse will then take some physical measurements including height and weight and a machine will measure the bone mass of your arm. The bone mass measurement involves taking an X ray of your forearm, is painless and will only take about five minutes. The exposure to X-rays during this measurement is minimal and will not affect your health in any way. Every six months when you return to the clinic for your repeat injection or re-supply of pills we will interview you and repeat the height, weight and bone mass measurement. The total length of the study is five years and we would want you to stay in the study for the whole of the five year period. If you know this will not be possible because you are planning to move away from the area in the next few years please inform the study nurse.

What if I want to stop using the injection/pill or withdraw from the study?

Participation in the study is entirely voluntary and you may withdraw at any time. This will not affect your future treatment at the clinic. All the information you give us will be treated in confidence. We will reimburse any travel costs you incur during the course of the study.

For more information on the study, the following people can be contacted:

Mags Beksinska / Fikile Mbatha, family planning Clinic, Commercial Road – 307 2781

SUBJECT INFORMATION SHEET
(15-19 age group, non-users of hormonal contraceptives)

INJECTABLE AND ORAL CONTRACEPTIVE BONE MASS STUDY

What is this study about?

The injectable contraceptives Depo Provera and Nuristerate and oral contraceptives (pills) have been used by women in this country for many years. The Reproductive Health Research Unit based in the Department of Obstetrics and Gynaecology of Baragwanath Hospital is undertaking a study to look at the effect of these contraceptives on the bones of the skeleton (bone mass).

What will be involved If I take part in this study?

We are looking for women volunteers aged between 15 and 19 who have never used the injection or the pill (hormonal contraceptives). If you decide to volunteer, A nurse will interview you to collect information on your medical history, contraceptive history, and other reproductive information including pregnancies and breastfeeding. The nurse will then take some physical measurements including height and weight and a machine will measure the bone mass of your arm. The bone mass measurement involves taking an X ray of your forearm, is painless and will only take about five minutes. The exposure to X-rays during this measurement is minimal and will not affect your health in any way. We will ask you to come to the clinic every six months for another interview and we will repeat the height, weight and bone mass measurement. The total length of the study is five years and we would want you to stay in the study for the whole of the five year period. If you know this will not be possible because you are planning to move away from the area in the next few years please inform the study nurse.

What if I want to start using the injection/pill or withdraw from the study?

Participation in the study is entirely voluntary and you may withdraw at any time. This will not affect your future treatment at the clinic. All the information you give us will be treated in confidence. We will reimburse any travel costs you incur during the course of the study.

For more information on the study, the following people can be contacted:

Mags Beksinska / Fikile Mbatha, family planning Clinic, Commercial Road – 307 2781

SUBJECT INFORMATION SHEET
(40-49 age group, users of hormonal contraceptives)

INJECTABLE AND ORAL CONTRACEPTIVE BONE MASS STUDY

What is this study about?

The injectable contraceptives Depo Provera and Nuristerate and oral contraceptives (pills) have been used by women in this country for many years. The Reproductive Health Research Unit based in the Department of Obstetrics and Gynaecology of Baragwanath Hospital is undertaking a study to look at the effect of these contraceptives on the bones of the skeleton (bone mass).

What will be involved If I take part in this study?

We are looking for women volunteers aged between 40 and 49 who are current users of Depo-Provera, Nur-isterate or oral contraceptives to take part in this study. If you decide to volunteer, A nurse will interview you to collect information on your medical history, contraceptive history, and other reproductive information including pregnancies and breastfeeding. The nurse will then take some physical measurements including height and weight and a machine will measure the bone mass of your arm. The bone mass measurement involves taking an X ray of your forearm, is painless and will only take about five minutes. The exposure to X-rays during this measurement is minimal. Many women begin to experience symptoms of menopause between the ages of 40 and 50 so we will also ask you some questions about any menopausal symptoms you may have experienced and take a blood test to check if you may be menopausal. Every six months when you return to the clinic for your repeat injection or re-supply of pills we will interview you and repeat the height, weight, bone mass measurement and blood test. The total length of the study is five years and we would want you to stay in the study for the whole of the five year period. If you know this will not be possible because you are planning to move away from the area in the next few years please inform the study nurse.

What if I want to stop using the injection/pill or withdraw from the study?

Participation in the study is entirely voluntary and you may withdraw at any time. This will not affect your future treatment at the clinic. All the information you give us will be treated in confidence. We will reimburse any travel costs you incur during the course of the study.

For more information on the study, the following people can be contacted:

Mags Beksinska / Fikile Mbatha family planning Clinic, Commercial Road – 307 2781

SUBJECT INFORMATION SHEET
(40-49 age group, non-hormonal contraceptive users)

INJECTABLE AND ORAL CONTRACEPTIVE BONE MASS STUDY

What is this study about?

The injectable contraceptives Depo Provera and Nuristerate and oral contraceptives (pills) have been used by women in this country for many years. The Reproductive Health Research Unit based in the Department of Obstetrics and Gynaecology of Baragwanath Hospital is undertaking a study to look at the effect of these contraceptives on the bones of the skeleton (bone mass).

What will be involved If I take part in this study?

We are looking for women volunteers aged between 40 and 49 who are not using the injection or the pill (hormonal contraceptives) to take part in this study. If you decide to volunteer, A nurse will interview you to collect information on your medical history, contraceptive history, and other reproductive information including pregnancies and breastfeeding. The nurse will then take some physical measurements including height and weight and a machine will measure the bone mass of your arm. The bone mass measurement involves taking an X ray of your forearm, is painless and will only take about five minutes. The exposure to X-rays during this measurement is minimal. Many women begin to experience symptoms of menopause between the ages of 40 and 49 so we will also ask you some questions about any menopausal symptoms you may have experienced and take a blood test to check if you may be menopausal. We will ask you to come to the clinic every six months for another interview and repeat the height, weight, bone mass measurement and blood test. The total length of the study is five years and we would want you to stay in the study for the whole of the five year period. If you know this will not be possible because you are planning to move away from the area in the next few years please inform the study nurse.

What if I want to start using the injection/pill or withdraw from the study?

Participation in the study is entirely voluntary and you may withdraw at any time. This will not affect your future treatment at the clinic. All the information you give us will be treated in confidence. We will reimburse any travel costs you incur during the course of the study.

For more information on the study, the following people can be contacted:

Mags Beksinska / Fikile Mbatha, family planning Clinic, Commercial Road – 307 2781

Zulu subject information sheet aged 15 – 19 age group users of hormonal contraceptives

ISAZISO MAYELANA NOCWANINGO (15 -19 iminyaka)

UCWANINGO NGESISINDO SAMATHAMBO OLUPHATHELENE NOHLELO
MNDENI OLUNJENGOMJOVO KANYE NAMAPHILISI.

INGABE LUMAYELANA NANI LULUCWANINGO/UPHANDO?

Izinhlobo zokuhlela imindeni ezinjengemijovo (depo provera, kanye namaphilisi) ziye zasetshenziswa ngabesifazane kuleli lakithi iminyaka eminingi. I-REPRODUCTIVE HEALTH RESEARCH UNIT exhumane ne Department Of Obstertric and Gynaecology ese Chris Hani Baragwanath Hospital yenza uphando mayelana nendlela izinhlobo zokuhlela imindeni ezithintana ngayo nesimo samathambo (isisindo samathambo).

KUXHOMEKANI UKUTHATHA KWAMI UHLANGOTHI KULOLUPHANDO?

Sifuna abantu besifazane abazozinikela ngokuzithandela abaphakathi kweminyaka engu 15 kuya kweyi-19 abafuna ukusebenzisa imijovo (depo provera, nur-isterate noma amaphilisi okuhlela) ukuba bangelele loluphando. Uma ufisa ukuzinikela ngokuzithandela, umhlengikazi uyobe esekubuza imibuzo ngempilo yakho/izifo, uhlelo mndeni owake walusebenzisa, imibuzo ephathelene nembeleko yakho kanye nokuncelisa. Umhlengikazi uyobe esekukala ubude, isisindo futhi umshini uyosetshenziswa ukukala isisindo sengalo yakho. Lokhu ukukalwa kwamathambo kubandakanya ukuhlolwa kwamathambo engalo (x-ray), akubuhlungu futhi kuthatha imizuzu emihlanu nje kuphela kanti akunangozi. Njalo nje emva kwezinyanga eziyisithupha uma usubuyela emtholampilo ukuzohlaba noma uzolanda amaphilisi akho okuhlela uyobuzwa imibuzo uphinde ukalwe ubude, isisindo kanye nesisindo samathambo futhi nokuhlolwa kwegazi mayelana nokuvala kwakho ungasayi esikhathini (menopause). Siyothanda ukukubona iminyaka emihlanu ucwaningo selulonke.

Kuyokwenzekani uma sengiyeka ukusebenzisa lolu hlelo mndeni noma ngihoxa kuloluphando?

Ukuthatha uhlangothi kuloluphando kungokuzithandela futhi ungasaxa noma yinini uma ungasafuni. Lokhu angeke kuphazamise ukusebenzisa kwakho umtholampilo ngokuzayo. Yonke into ositshela yona iyogcinwa njengeyimfihlo. Futhi siyobhekana nezindleko zakho zokugibela eziyokwenzakala phakathi naloluphando.

Uma ufuna ukwazi kabanzi ngalolucwaningo/uphando, thintana nalaba abalandelayo:

Mags Beksinska / Fikile Mbatha Family Planning Clinic, Commercial Road – 307 2781

Zulu subject information sheet – age 15-19 non - users of hormonal contraceptives
ISAZISO MAYELANA NOCWANINGO
(15 -19 iminyaka)

UCWANINGO NGESISINDO SAMATHAMBO OLUPHATHELENE NOHLELO
MNDENI OLUNJENGOMJOVO KANYE NAMAPHILISI.

INGABE LUMAYELANA NANI LULUCWANINGO/UPHANDO

Izinhlobo zokuhlela imindeni ezinjengemijovo (depo provera, kanye namaphilisi) ziye zasetshenziswa ngabesifazane kuleli lakithi iminyaka eminingi. I-REPRODUCTIVE HEALTH RESEARCH UNIT exhumane ne Department Of Obstetric and Gynaecology ese Chris Hani Baragwanath Hospital yenza uphando mayelana nendlela izinhlobo zokuhlela imindeni ezithintana ngayo nesimo samathambo (isisindo samathambo).

KUXHOMEKANI UKUTHATHA KWAMI UHLANGOTHI KULOLUPHANDO?

Sifuna abantu besifazane abazozinikela ngokuzithandela abaphakathi kweminyaka engu 15 kuya kweyi-19 abangazange basebenzisa izinhlobo zohlelo mndeni kungaba imijovo noma amaphilisi ukuba bangelele lolu phando. Uma ufisa ukuzinikela ngokuzithandela, umhlengikazi uyobe esekubuza imibuzo ngempilo yakho/izifo, uhlelo mndeni owake walusebenzisa, imibuzo ephathelene nembeleko yakho kanye nokuncelisa. Umhlengikazi uyobe esekukala ubude, isisindo futhi umshini uyosetshenziswa ukukala isisindo sengalo yakho. Lokhu ukukalwa kwamathambo kubandakanya ukuhlolwa kwamathambo engalo (x-ray), akubuhlungu futhi kuthatha imizuzu emihlanu nje kuphela kanti akunangozi. Njalo nje emva kwezinyanga eziyisithupha uma usubuyela emtholampilo ukuzohlaba noma uzolanda amaphilisi akho okuhlela uyobuzwa imibuzo uphinde ukalwe ubude, isisindo kanye nesisindo samathambo futhi nokuhlolwa kwegazi mayelana nokuvala kwakho ungasayi esikhathini (menopause). Siyothanda ukukubona iminyaka emihlanu ucwaningo selulonke.

Kuyokwenzekani uma sengiyeka ukusebenzisa lolu hlelo mndeni noma ngihoxa kuloluphando?

Ukuthatha uhlangothi kuloluphando kungokuzithandela futhi ungahoxa noma yinini uma ungasafuni. Lokhu angeke kuphazamise ukusebenzisa kwakho umtholampilo ngokuzayo. Kanti uyokhuleka ukusebenzisa uhlelo mndeni phakathi naloluphando uma usufisa ukuhlela. Yonke into ositshela yona iyogcinwa njengeyimfihlo. Futhi siyobhekana nezindleko zakho zokugibela eziyokwenzakala phakathi naloluphando.

Uma ufuna ukwazi kabanzi ngalolucwaningo/uphando, thintana nalaba abalandelayo:

Mags Beksinska / Fikile Mbatha Family Planning Clinic, Commercial Road – 307 2781

Zulu subject information sheet 40 – 49 age group users of hormonal contraceptives
ISAZISO MAYELANA NOCWANINGO
40-49 IMINYAKA

UCWANINGO NGESISINDO SAMATHAMBO OLUPHATHELENE NOHLELO MNDENI
OLUNJENGOMJOVO KANYE NAMAPHILISI

INGABE LUMAYELANA NANI LOLUCWANINGO/UPHANDO

Izinhlobo zokuhlela imindeni ezinjengemijovo (depo provera, kanye namaphilisi) ziye zasetshenziswa ngabesifazane kuleli lakithi iminyaka eminingi. I-REPRODUCTIVE HEALTH RESEARCH UNIT exhumane ne Department of Obstetric and Gynaecology ese Chris Hani Baragwanath Hospital yenza uphando mayelana nendlela izinhlobo zokuhlela imindeni ezithintana ngayo nesimo samathambo (isisindo samathambo).

KUXHOMEKANI UKUTHATHA KWAMI UHLANGOTHI KULOLUPHANDO?

Sifuna abantu besifazane abazozinikela ngokuzithandela abaphakathi kweminyaka engu 40 kuya kweyi-49 abasebenzisa imijovo (depo provera, nur-isterate) noma amaphilisi okuhlela ukuba bangenele lolu phando. Uma ufisa ukuzinikela ngokuzithandela, umhlengikazi uyobe esekubuza imibuzo ngempilo yakho/izifo, uhlelo mndeni owake walusebenzisa, imibuzo ephathelene nembeleko yakho kanye nokuncelisa. Umhlengikazi uyobe esekukala ubude, isisindo futhi umshini uyosetshenziswa ukukala isisindo sengalo yakho. Lokhu ukukalwa kwamathambo kubandakanya ukuhlolwa kwamathambo engalo (x-ray), akubuhlungu futhi kuthatha imizuzu emihlanu nje kuphela kanti akunangozi.

Abesifazane abaningi baqala ukuvaleka ukuya esikhathini (MENOUPAUSE) beneminyaka engu 42 kuya kweyi-49, futhi sizokubuza nemibuzo mayelana nezimpawu owake wahlangabezana nazo ngesikhathi uvaleka ukuya esikhathini bese sithatha igazi ukuhlola ukuthi ingabe sewusezingeni lokuvaleka ungasayi esikhathini na. Njalo nje emva kwezinyanga eziyisithupha uma usubuyela emtholampilo ukuzohlaba noma uzolanda amaphilisi akho okuhlela uyobuzwa imibuzo uphinde ukalwe ubude, isisindo kanye nesisindo samathambo futhi nokuhlolwa kwegazi mayelana nokuvaleka kwakho ungasayi esikhathini. Siyothanda ukukubona iminyaka emihlanu ucwaningo selulonke.

Kuyokwenzekani uma sengiyeka ukusebenzisa loluhlelo mndeni noma ngihoxa kuloluphando?

Ukuthatha uhlangothi kuloluphando kungukuzithandela futhi ungahoxa noma yinini uma ungasafuni. Lokhu angeke kuphazamise ukusebenzisa kwakho umtholampilo ngokuzayo. Kanti uyokhululeka ukusebenzisa uhlelo mndeni phakathi naloluphando uma usufisa ukuhlela. Yonke into ositshela yona iyogcinwa njengeyimfihlo. Siyobe futhi sesibhekana nezindleko zakho zokugibela eziyokwenzakala phakathi naloluphando.

Uma ufuna ukwazi kabanzi ngalolucwaningo/uphando, thintana nalaba abalandelayo.
Mags Beksinska / Fikile Mbatha Family Planning Clinic, Commercial Road – 031 307 2781

Zulu subject information sheet aged 40 – 49 non –users of hormonal contraceptives

ISAZISO MAYELANA NOCWANINGO 40-49 IMINYAKA

UCWANINGO NGESISINDO SAMATHAMBO OLUPHATHELENE NOHLELO
MNDENI OLUNJENGOMJOVO KANYE NAMAPHILISI

INGABE LUMAYELANA NANI LOLUCWANINGO/UPHANDO

Izinhlobo zokuhlela imindeni ezinjengemijovo (Depo provera, kanye namaphilisi) ziye zasetshenziswa ngabesifazane kuleli lakithi iminyaka eminingi. I- REPRODUCTIVE HEALTH RESEARCH UNIT exhumane ne Department of Obstetric and Gynaecology ese Chris Hani Baragwanath Hospital yenza uphando mayelana nendlela izinhlobo zokuhlela imindeni ezithintana ngayo nesimo samathambo (isisindo samathambo).

KUXHOMEKANI UKUTHATHA KWAMI UHLANGOTHI KULOLUPHANDO?

Sifuna abantu besifazane abazozinikela ngokuzithandela abaphakathi kweminyaka engu 40 kuya kweyi-49 abasebenzisa izinhlobo zokuhlela umndeni ezinjengemijovo (depo provera, nur-isterate noma amaphilisi okuhlela) ukuba bangenele lolu phando. Uma ufisa ukuzinikela ngokuzithandela, umhlengikazi uyobe esekubuza imibuzo ngempilo yakho/izifo, uhlelo mndeni owake walusebenzisa, imibuzo ephathelene nembeleko yakho kanye nokuncelisa. Umhlengikazi uyobe esekukala ubude, isisindo futhi umshini uyosetshenziswa ukukala isisindo sengalo yakho. Lokhu ukukalwa kwamathambo kubandakanya ukuhlolwa kwamathambo engalo (x-ray), akubuhlungu futhi kuthatha imizuzu emihlanu nje kuphela kanti akunangozi.

Abesifazane abaningi baqala ukuvaleka ukuya esikhathini (MENOUPAUSE) beneminyaka engu 42 kuya kweyi-49, futhi sizokubuza nemibuzo mayelana nezimpawu owake wahlangabezana nazo ngesikhathi uvaleka ukuya esikhathini bese sithatha igazi ukuhlola ukuthi ingabe sewusezingeni lokuvaleka ungasayi esikhathini na. Njalo nje emva kwezinyanga eziyisithupha uma usubuyela emtholampilo ukuzohlaba noma uzolanda amaphilisi akho okuhlela uyobuzwa imibuzo uphinde ukalwe ubude, isisindo kanye nesisindo samathambo futhi nokuhlolwa kwegazi mayelana nokuvaleka kwakho ungasayi esikhathini. Siyothanda ukukubona iminyaka emihlanu ucwaningo selulonke.

Kuyokwenzekani uma sengiyeka ukusebenzisa lolu hlelo mndeni noma ngihoxa kuloluphando?

Ukuthatha uhlangothi kuloluphando kungokuzithandela futhi ungahoxa noma yinini uma ungasafuni. Lokhu angeke kuphazamise ukusebenzisa kwakho umtholampilo ngokuzayo. Kanti uyokhululeka ukusebenzisa uhlelo mndeni phakathi naloluphando uma usufisa ukuhlela. Yonke into ositshela yona iyogcinwa njengeyimfihlo. Siyobe futhi sesibhekana nezindleko zakho zokugibela eziyokwenzakala phakathi naloluphando.

**Uma ufuna ukwazi kabanzi ngalolucwaningo/uphando, thintana nalaba abalandelayo:
Mags Beksinska / Fikile Mbatha Family Planning Clinic, Commercial Road – 307 2781**

INFORMED CONSENT
INJECTABLE AND ORAL CONTRACEPTIVE BONE MASS STUDY
(Age 15-19 hormonal contraceptive users)

I have been informed that this research study looks at the possible effect of some contraceptives on the microscopic structure and mineral content of skeletal bones (bone mass) of the body, and that some contraceptives may lower the bone mass during use and that the bone mass usually returns to normal after stopping use.

I understand that I will be interviewed and have a medical history taken and complete a questionnaire. In addition an examination will include a weight, height and bone mass measurement. Every six months when I come back to the clinic for another injection/supply of pills I will be interviewed again and the examinations will be repeated. I understand that the total length of the study is five years.

I further more have been informed that the bone mass measurement exposes me to a minimal dose of x-rays that will not affect my health, and that if I fall pregnant at any time during the follow-up period I will no longer be able to continue in the study.

I understand that there is no direct benefit to me from participation in this research. I can stop or change my method of contraception at any time. I can withdraw from the study at any time and this will not, in any way, affect my future treatment at the clinic.

I have had the study explained to me and I have read the information sheet and this form or had it read to me. I have had the opportunity to ask questions on the study and what is involved if I take part.

I agree voluntarily to participate in this study.

Signed:_____

Witness:_____

INFORMED CONSENT
INJECTABLE AND ORAL CONTRACEPTIVE BONE MASS STUDY
(Age 15-19 non hormonal contraceptive users)

I have been informed that this research study looks at the possible effect of some contraceptives on the microscopic structure and mineral content of skeletal bones (bone mass) of the body, and that some contraceptives may lower the bone mass during use and that the bone mass usually returns to normal after stopping use.

I understand that I will be interviewed and have a medical history taken and complete a questionnaire. In addition an examination will include a weight, height and bone mass measurement. I will be asked to come to the clinic every six months to be interviewed again and the examinations will be repeated. I understand that the total length of the study is five years.

I further more have been informed that the bone mass measurement exposes me to a minimal dose of x-rays that will not affect my health, and that if I fall pregnant at any time during the follow-up period I will no longer be able to continue in the study.

I understand that there is no direct benefit to me from participation in this research. I can stop or change my method of contraception at any time. I can withdraw from the study at any time and this will not, in any way, affect my future treatment at the clinic.

I have had the study explained to me and I have read the information sheet and this form or had it read to me. I have had the opportunity to ask questions on the study and what is involved if I take part.

I agree voluntarily to participate in this study.

Signed:_____

Witness:_____

Place:_____

Date:_____

INFORMED CONSENT
INJECTABLE AND ORAL CONTRACEPTIVE BONE MASS STUDY
(Age 40-49 hormonal contraceptive users)

I have been informed that this research study looks at the possible effect of some contraceptives on the microscopic structure and mineral content of skeletal bones (bone mass) of the body, and that some contraceptives may lower the bone mass during use and that the bone mass usually returns to normal after stopping use.

I understand that I will be interviewed and have a medical history taken and complete a questionnaire. In addition an examination will include a blood test, weight, height and bone mass measurement. Every six months when I come back to the clinic for another injection/supply of pills I will be interviewed again and the examinations will be repeated. I understand that the total length of the study is five years.

I further more have been informed that the bone mass measurement exposes me to a minimal dose of x-rays that will not affect my health, and that if I fall pregnant at any time during the follow-up period I will no longer be able to continue in the study.

I understand that there is no direct benefit to me from participation in this research. I can stop or change my method of contraception at any time. I can withdraw from the study at any time and this will not, in any way, affect my future treatment at the clinic.

I have had the study explained to me and I have read the information sheet and this form or had it read to me. I have had the opportunity to ask questions on the study and what is involved if I take part.

I agree voluntarily to participate in this study.

Signed:_____

Witness:_____

Place:_____

Date:_____

INFORMED CONSENT
INJECTABLE AND ORAL CONTRACEPTIVE BONE MASS STUDY
(Age 40-49 non hormonal contraceptive users)

I have been informed that this research study looks at the possible effect of some contraceptives on the microscopic structure and mineral content of skeletal bones (bone mass) of the body, and that some contraceptives may lower the bone mass during use and that the bone mass usually returns to normal after stopping use.

I understand that I will be interviewed and have a medical history taken and complete a questionnaire. In addition an examination will include a weight, height and bone mass measurement. I will be asked to return to the clinic every six months to be interviewed again and the examinations will be repeated. I understand that the total length of the study is five years.

I further more have been informed that the bone mass measurement exposes me to a minimal dose of x-rays that will not affect my health, and that if I fall pregnant at any time during the follow-up period I will no longer be able to continue in the study.

I understand that there is no direct benefit to me from participation in this research. I can stop or change my method of contraception at any time. I can withdraw from the study at any time and this will not, in any way, affect my future treatment at the clinic.

I have had the study explained to me and I have read the information sheet and this form or had it read to me. I have had the opportunity to ask questions on the study and what is involved if I take part.

I agree voluntarily to participate in this study.

Signed:_____

Witness:_____

Place:_____

Date:_____

Zulu Informed consent form
Age group 15 -19 hormonal contraceptives users

ISIVUMELEWANO EMVA KWENCAZELO

UPHANDO MAYELANA NESISINDO SAMATHAMBO OKUTHINTANA NGA KHO
NEZINHLELO MNDENI EZINJENGEMIJOVO KANYE NAMAPHILISI.
(15 - 19 yeminyaka yabangasebenzisi izinhlobo zokuhlela umndeni)

Ngiyewisisa ukuthi lolucwaningo/uphando lubheke indlela ezinye izinhlobo zokuhlela ezithinta ngayo isimo samathambo. Ngiyaqonda futhi ukuthi ezinye izinhlelo mndeni zehlisa isisindo samathambo ngesikhathi zisasetshenziswa kanti isisindo sibuyela endaweni uma seziyekiwe ukusetshenziswa.

Ngiyaqonda nokuthi ngiyobe sengibuzwa imibuzo mayelana nempilo yami futhi ngigcwalise ne fomu. Ngaphezu kwalokho ukuhlolwa komzimba/kwempilo kuyobandakanya ukukalwa kwesisindo, ubude kanye nesisindo samathambo. Ngiyaqonda nokuthi lonke lolucwaningo/uphando luyothatha imiyaka emihlanu seyiyonke.

Ngiyewisisa nokuthi lolukalo lwamathambo luzokwenza nokuthi ngingene emshinini wokuhlola amathambo (x-ray). Lokhu akubuhlungu futhi akunabungozi futhi kuthatha isikhashana nje.

Ngiyewazwa nokuthi uma ngingakhulelwa phakathi nalophando angeke ngisakhona ukuqhubeka ngiyingxenywe yaloluphando.

Ngiyaqonda nokuthi akukho nzuzo ngokuqondile engiyoyizuza ngokungenela loluphando. Ngingayeka/hoxa noma ngishintshe uhlobo lohlelo mndeni noma yinini uma ngingasathandi. Ngingahoxa noma yinini ekubeni yingxenywe yalolucwaningo/ uphando futhi lokhu, angeke kuphazamise ukusebenzisa kwami umtholampilo ngokuzayo.

Ngizwile konke okushiwo ngalolucwaningo futhi ngizifunde ipheshana lesaziso noma bangifunde. Ngitholile nethuba lukuzibuzela imibuzo mayelana nalolucwaningo nokuthi kuxhomekeni ukuthatha kwami uhlangathi kuloluphando/ucwaningo.

Ngizivumela ngokuzithandela ukungenela loluphando/ucwaningo.

Isayinwe

Ufakazi

Indawo

Usuku

Zulu informed consent form
Age 15-19 age group non-hormonal contraceptive users

ISIVUMELEWANO EMVA KWENCAZELO

UPHANDO MAYELANA NESISINDO SAMATHAMBO OKUTHINTANA NGAkho
NEZINHLELO MNDENI EZINJENGEMIJOVO KANYE NAMAPHILISI.
(15-19 yeminyaka)

Ngiezwisisa ukuthi lolucwaningo/uphando lubheke indlela ezinye izinhlobo zokuhlala ezithinta ngayo isimo samathambo. Ngiaqonda futhi ukuthi ezinye izinhlelo mndeni zehlisa isisindo samthambo ngesikhathi zisasetshenziswa kanti isisindo sibuyela esimweni uma seziyekiwe ukusetshenziswa.

Ngiaqonda nokuthi ngiyobe sengibuzwa imibuzo mayelana nempilo yami futhi ngigcwalise ne fomu. Ngaphezu kwalokho ukuhlola komzimba/kwempilo kuyobandakanya ukukalwa kwesisindo, ubude kanye nesisindo samathambo. Njalo nje ezinyangeni eziyisithupha uma sengibuyela emtholampilo ukuzolaba noma ukulanda amaphilisi ngiyophinde ngibuzwe imibuzo, futhi ngihloliwe ngokwempilo. Ngiaqonda nokuthi lonke lolucwaningo/uphando luyothatha iminyaka emihlanu selulomke.

Ngiezwisisa nokuthi lolukalo lwamathambo luzokwenza nokuthi ngingene emshinini wokuhlola amathambo i (X-ray). Lokhu akubuhlungu futhi akunabungozi futhi kuthatha isikhashana nje.

Ngieywa nokuthi uma ngingakhulelwa phakathi nalophando, angeke ngisaqhubeka ngiingxenye yaloluphando.

Ngiaqonda nokuthi akukho nzuzo ngokuqondile engiyoyizuza ngokungenela loluphando. Ngingayeka/hoxa noma ngiyeke ukusebenzisa uhlelo mndeni noma yinini uma ngingasathandi. Ngingahoxa noma yinini futhi lokhu, angeke kuphazamise ukusebenzisa kwami umtholampilo ngokuzayo.

Ngizwile konke okushiwo ngalulucwaningo futhi ngizifundele ipheshana lesaziso noma bangifundele. Ngitholile nethuba lukuzibuzela imibuzo mayelana nalolucwaningo nokuthi kuxhomekeni ukuthatha kwami uhlangathi kuloluphando/ucwaningo.

Ngizivumela ngokuzithandela ukungenela loluphando/ucwaningo.

Isayinwe

Ufakazi

Indawo

Usuku

Zulu informed consent form
Age group 40-49 hormonal contraceptive user

ISIVUMELEWANO EMVA KWENCAZELO

UPHANDO MAYELANA NESISINDO SAMATHAMBO OKUTHINTANA NGA KHO
NEZINHLELO MNDENI EZINJENGEMIJOVO KANYE NAMAPHILISI.
(40-49 yeminyaka)

Ngieywisisa ukuthi lolucwaningo/uphando lubheke indlela ezinye izinhlobo zokuhlela ezithintana ngayo namathambo. Ngiaqonda futhi ukuthi ezinye izinhlelo mndeni zehlisa isisindo samathambo ngesikhathi zisasetshenziswa kanti isisindo sibuyela endaweni uma seziyekiwe ukusetshenziswa.

Ngiaqonda nokuthi ngiyobe sengibuzwa imibuzo mayelana nempilo yami futhi ngigcwalise ne fomu. Ngaphezu kwalokho ukuhlolwa komzimba/kwempilo kuyobandakanya ukuhlolwa kwegazi (ukuze sikwazi ukuthola ukuthi ingabe sewusezingeni lokuvala ungasayi esikhathini,) ukukalwa kwesisindo, ubude kanye nesisindo samathambo. Ezinyangeni eziyisithupha uma sengibuyela emtholampilo ukuzohlaba noma ukulanda amaphilisi okuhlela ngiyobe sengibuzwa futhi imibuzo, ukuhlolwa ngempilo/umzimba kuyophindwa. Ngiaqonda nokuthi lonke lolucwaningo/uphando luyothatha imiyaka emihlanu seyiyonke.

Ngieywisisa nokuthi lolukalo lwathambo luzokwenza nokuthi ngingene emshinini wokuhlola amathambo (x-ray). Lokhu akubuhlungu futhi akunabungozi futhi kuthatha isikhashana nje.

Ngieywa nokuthi uma ngingakhulelwa phakathi nalophando angeke ngisakhona ukuqhubeka ngiyingxenye yaloluphando.

Ngiaqonda nokuthi akukho nzuzo ngokuqondile engiyoyizuza ngokungenela loluphando. Ngingayeka/ngihoxe noma ngiyeke ukusebenzisa uhlelo mndeni noma yinini uma ngingasathandi. Ngingahoxa noma yinini futhi lokhu, angeke kuphazamise ukusebenzisa kwami umtholampilo ngokuzayo.

Ngizwile konke okushiwo ngalulucwaningo futhi ngizifundele ipheshana lesaziso noma bangifundele. Ngitholile nethuba lukuzibuzela imibuzo mayelana nalolucwaningo nokuthi kuxhomekeni ukuthatha kwami uhlangothi kuloluphando/ucwaningo.

Ngizivumela ngokuzithandela ukungenela loluphando/ucwaningo.

Isayinwe

Ufakazi

Indawo

Usuku

Zulu informed consent form
Age 40-49 non hormonal contraceptive users

ISIVUMELEWANO EMVA KWENCAZELO

UPHANDO MAYELANA NESISINDO SAMATHAMBO OKUTHINTANA NGAKHO
NEZINHLELO MNDENI EZINJENGEMIJOVO KANYE NAMAPHILISI.
(40-49 yeminyaka abangasebenzisi izinto zokuhlela/zokuvimbela inzalo)

Ngiezwisisa ukuthi lolucwaningo/uphando lubheke indlela ezinye izinhlobo zokuhlela ezithintana ngayo namathambo. Ngiaqonda futhi ukuthi ezinye izinhlelo mndeni zehlisa isisindo samthambo ngesikhathi zisasetshenziswa kanti isisindo sibuyela endaweni uma seziyeke ukusetshenziswa.

Ngiaqonda nokuthi ngiyobe sengibuzwa imibuzo mayelana nempilo yami futhi ngicwalise ne fomu. Ngaphezu kwalokho ukuhlolwa komzimba/kwempilo kuyobandakanya ukuhlolwa kwegazi, (ukuze kutholakala ukuthi ingabe sengisezingeni lokuvaleka ngingasayi esikhathini) ukukalwa kwesisindo, ubude kanye nesisindo samathambo. Ngiaqonda nokuthi lonke lolucwaningo/uphando luyothatha imiyaka emihlanu seiyonke.

Ngiezwisisa nokuthi lolukalo lwamathambo luzokwenza nokuthi ngingene emshinini wokuhlola amathambo (X-ray). Lokhu akunabungozi futhi kuthatha isikhashana nje.

Ngieywa nokuthi uma ngingakhulelwa phakathi nalophando angeke ngisakhona ukuqhubeka ngiyingxenywe yaloluphando.

Ngiaqonda nokuthi akukho nzuzo ngokuqondile engiyoyizuza ngokungenela loluphando. Ngingayeka/ngihoxe noma ngiyeke ukusebenzisa uhlelo mndeni noma yinini uma ngingasathandi. Ngingahoxa noma yinini futhi lokhu, angeke kuphazamise ukusebenzisa kwami umtholampilo ngokuzayo.

Ngizwile konke okushiwo ngalulucwaningo futhi ngizifunde ipheshana lesaziso noma bangifunde. Ngitholile nethuba lukuzibuzela imibuzo mayelana nalolucwaningo nokuthi kuxhomekeni ukuthatha kwami uhlangathi kuloluphando/ucwaningo.

Ngizivumela ngokuzithandela ukungenela loluphando/ucwaningo.

Isayinwe

Ufakazi

Indawo

Usuku

Informed consent for spine/hip/femoral neck DEXA scan sub-study

**INFORMED CONSENT
INJECTABLE AND ORAL CONTRACEPTIVE BONE MASS STUDY**

I have been informed that the bone mass research study that there maybe some possible effect of some contraceptives on the microscopic structure and mineral content of skeletal bones (bone mass) of the body, and that some contraceptives may lower the bone mass during use and that the bone mass usually returns to normal after stopping use.

I understand that I will have one additional bone mass measurement to the six-monthly forearm measurement at Commercial city clinic. This additional measurement will be done at the Westridge Medical centre in Durban. This measurement will be of the hip and spine. I have been informed that the bone mass measurement will expose me to a minimal dose of x-rays that will not affect my health.

I have had the study explained to me and I have read the information sheet and this form or had it read to me. I have had the opportunity to ask questions on the study and what is involved if I agree to take part in this extra component of the study.

I agree voluntarily to participate in this study.

Signed:_____

Witness:_____

Place:_____

Date:_____

BONE MASS STUDY INFORMATION SHEET SEPT 2005

Dear participant

We would like to thank you for taking part in the bone mass study for the last five years. The study has now entered its 6th and final year of follow-up. We have so far measured the bone mineral density of your forearm. There is some concern that this site is not as sensitive to the effects of contraception. The World Health Organisation who fund the study have asked us to measure the hip and spine of a number of our participants. We do not have the equipment to do this at commercial city clinic, however in our main offices in Westridge there is a group of specialist diagnostic radiologists (Jackpersad and partners inc) where this could be done. This would be a one off measurement and would require an additional visit. The study nurse would be present and the scan will take about 1 minute to conduct. The equipment is similar to that used for your current measurement but we will ask you this time to lie on a bed. The measurement uses similar equipment to that used at commercial city clinic which is safe and uses very low radiation. You will not feel anything during the measurement. We have drafted an additional consent form for you to read and sign if you are willing to volunteer for this component of the study. You will receive an additional R50 for this visit to cover your transport costs. The study nurse Mrs Fikile Mbatha will explain what is involved and will book an appointment for you at a convenient time.

Kind regards

Mags Beksinska, Study manager Fikile Mbatha , Study Nurse co-ordinator

Zulu informed consent for spine/hip/femoral neck DEXA scan sub-study

ISIVUMELWANO EMVA KWENCAZELO

Uphando/ucwaningo maqondana nesisindo samathambo, nokusebenzisa izinhlelo-mndeni ezinjengemijovo kanye namaphilisi.

Ngichazeliwe, ngazwisisa ngokuthintana kwesisindo samathambo, nokusebenza kwezinhlelo-mndeni ezicutshini eziyinhlanganisela ezenza ithambo lomuntu.

Kuzwakele futhi ukuthi ezinye izinhlobo zezinhlela-mndeni zenza isisindo sethambo lithi ukwehla kancane; kodwa uma umuntu eseyekile ukuhlela sibuye (isisindo) sibuyele njenga njalo umuntu engakaqali umhlela-mndeni.

Ngiyavuma futhi ukuthatha esinye isisindo samathambo phezulu kwalezi ebengizenza njalo emva kwezinyanga eziyisithupha e **Commercial City Clinic**. Umahluko walesi ukuthi sona sizokwenzelwa e **Westridge Medical Centre** khona lapha e**Thekwini**.

Sizothatha isisindo samathambo; amanyongo nomqolo. Mincane imisebe ye Xray engena kimi futhi akunabungozi empilweni yami.

Ngichazelwe nganeliseka ngocwaningo, futhi ngiyifundile incazelo ngalo, ngafundiswa ngaqonda kahle. Ngithole ithuba ngabuza imibuzo yami yaphenduleka.

Ngiyavuma ngokuphelele ukuzibandakanya nokwenzekayo ngololuhlobo lwocwaningo.

Ovumayo: _____

Ufakazi: _____

Indawo: _____

Usuku: _____

CONFIDENTIAL ADDRESS FORM

If you stop using the injection or you do not return to the clinic for any reason we would like to be able to contact you to find out why? We will be keeping a separate register with your name and telephone number if you have one. It will not be possible for anybody to link your name, etc. with your questionnaires

☐

Can we contact you? (1=yes; 2=no)

Home address _____

Home tel no _____

☐

Work tel no _____

☐

Name of contact _____

His/Her tel no _____

If we need to get in touch with you would you prefer we contacted you at work or home?
(Tick in home or work box above)

APPENDIX 2

QUESTIONNAIRES

- SCREENING QUESTIONNAIRE (SCR)
- ADMISSION QUESTIONNAIRE (ADM)
- EXAMINATION QUESTIONNAIRE (EXM)
- FOLLOW-UP QUESTIONNAIRE (FOL)
- DISCONTINUATION QUESTIONNAIRE (DIS)
- FOLLOW-UP NON-ATTENDANCE QUESTIONNAIRE (NON)

REPRODUCTIVE HEALTH RESEARCH UNIT	INJECTABLE AND ORAL CONTRACEPTIVE BONE MASS STUDY SCREENING QUESTIONNAIRE	SCR Page.1
--	--	-----------------------

Screening number

--	--	--

Date of interview

--	--	--	--	--

Day

Month

Year

1. How old were you at your last birthday?	AGE IN COMPLETED YEARS		
2. In what month and year were you born?	MONTH		YEAR
3. Have you ever used a hormonal method of contraception before? (pill or injection) (15-19 age group only)		NO	YES
4. Are you still menstruating? (40-49 age group only)			
5. Are you currently breastfeeding?			
6. Have you breastfed in the last six months?			
6. Have you been pregnant in the last six months?			
8. Have you had a hysterectomy or oophrectomy?			
9. Are you planning to move out of the area in the next five years?			
10. Does subject have a history of any of the following? Tick box that applies		NO	YES
a) Liver disease (including chronic hepatitis)			
b) Diabetes mellitus			
c) Chronic kidney disease			
d) Thyroid condition or disease			
e) Parathyroid condition or disease			
f) Cancer			
g) Pituitary disease			
h) Sarcoidosis			
i) Bed rest for 6 weeks or longer in the last 12 months			
j) Bone disease (including rickets)			
k) Bone fracture in the last year			
l) Weight loss (5kg or more in the last six months)			
m) Chronic diarrhoea (in the last six months)			
n) Lymphadenopathy			
o) Tuberculosis			

REPRODUCTIVE HEALTH RESEARCH UNIT	INJECTABLE AND ORAL CONTRACEPTIVE BONE MASS STUDY SCREENING QUESTIONNAIRE	SCR Page.2
--------------------------------------	---	---------------

Screening number

12. Are you currently taking, or have you in the past taken for more than 3 months, any of the following medications?	YES	NO
a) Anticonvulsants	☐	☐
b) Systematic corticosteroids	☐	☐
c) Drugs for hypo- or hyper-thyroidism	☐	☐
d) Drugs for hypo- or hyper-parathyroidism	☐	☐
e) Vitamin D or C	☐	☐
f) Thiazide diuretics	☐	☐
g) Calcium supplements (except during pregnancy or lactation)	☐	☐
h) Antacids	☐	☐
IF THE ANSWER TO ANY OF THE ABOVE ARE YES, EXCLUDE SUBJECT FROM THIS STUDY		
13. a) (45 –49) Which contraceptive method are you currently using?		
b) (15 –19) Which method have you started taking?		

NONE.....0		
DEPO.....1		
NURISTERATE.....2		
COMBINED ORAL PILLS.....3		
PROGESTIN- ONLY PILLS.....4		
IUD.....5		
FEMALE STERILIZATION.....6		
BARRIER METHODS.....7		
NATURAL METHODS.....8		
MALE STERILIZATION.....9		
OTHER.....99	☐	☐
	YES	NO
14. Screened for normal range but does not wish to take part		
Subject study number		
Interviewers Name:		
Signature:	-----	

REPRODUCTIVE HEALTH RESEARCH UNIT	INJECTABLE AND ORAL CONTRACEPTIVE BONE MASS STUDY ADMISSION QUESTIONNAIRE	ADM Page 1
--	--	-----------------------

Subject study number Date of interview
Day Month Year

1. How old were you at your last birthday?

AGE IN COMPLETED YEARS

2. What is your ethnic group?

BLACK.....1
COLOURED.....2
INDIAN.....3

3. What is the highest level of education
That you have reached?

STANDARD

GRADE

POST MATRIC _____

EMPLOYED FULL / PART TIME.....1

EMPLOYED CASUAL.....2

UNEMPLOYED..... 3

HOUSEWIFE / AT HOME.....4

STUDENT.....5

SCHOLAR.....6

4. What is your household income per month?

R

5. What is your current relationship /
Marital status?

MARRIED (TRAD OR LEGAL).....1

COHABITING.....2

REG PARTNER (NOT COHAB).....3

CASUAL PARTNER.....4

SINGLE (NO PARTNER).....5

7a. Do you have a long standing illness?

NO.....1 Q8

YES.....2 Part b

7b. If yes, can you tell us what this illness is?

LIST DISEASE / S

ICD 10

ICD 10

8a. Are you currently taking any
Long term medication?

NO.....1

YES.....2 -----Part

8b. If yes, what medication are you taking

LIST

We would like to ask you some questions about
your Menstrual cycle and any pregnancies you have had.

9. How old were you when you had your first Menstrual
period?

YEARS

10. Do you menstruate regularly?

NO.....1

YES.....2

**REGULAR CYCLE DEFINED AS A PERIODIC
CYCLICAL BLEEDING OCCURRING AT
INTERVALS BETWEEN 21-35 DAYS**

11. Average length of the last three spontaneous
menstrual cycles

DAYS

12. Since the age of 16, have you ever missed
a menstrual period for one year or more
(excluding the time you were pregnant,

breastfeeding or used DMPA Or NETEN)?

NO 1-----Q14

YES 2-----Q14

REPRODUCTIVE HEALTH RESEARCH UNIT	INJECTABLE AND ORAL CONTRACEPTIVE BONE MASS STUDY ADMISSION QUESTIONNAIRE	ADM Page 2
---	---	---------------

Subject study number
Q13 AGE GROUP 1 ONLY (OLDER WOMEN)

Have you experienced any of the
Following in the last three months

YES NO

a) Hot flushes ☐ ☐

b) Night sweats ☐ ☐

c) Skin changes ☐ ☐

c) Dry vagina ☐ ☐

13. How many pregnancies have you
Had, including miscarriages and abortions?

NO OF PREGNANCIES

IF NEVER BEEN PREGNANT GO TO Q17

14. How many times have you given birth? ☐
NO OF BIRTHS

Did you breast feed any of your children?

NO.....1-----Q17
YES.....2

Children	Month and Year of birth	Total breast feeding (months)
1 st child		
2 nd child		
3 rd child		
4 th child		
5 th child		
6 th child		

*We would like to ask you some more details about
the Contraceptive method you may be using now and those
that you have used in the past and how long you used
them for.*

17. Which contraceptive method are you
currently using? ☐

NONE.....0

DEPO.....1

NURISTERATE.....2

COMBINED ORAL PILLS.....3

PROGESTIN-ONLY PILLS.....4

IUD.....5

FEMALE STERILIZATION.....6

BARRIER.....7

NATURAL METHODS.....8

MALE STERILIZATION..... 9

OTHER..... 99

18. Date of last injection

19a. Do you know the name of the brand
of pills you are now using?

ask to see packet or check clinic card records

BRAND NAME ☐

Nordette.....2

Ovral.....3

Marvalon.....4

19b. Time in pill cycle ☐

taking active pill 1

7 day interval.....2

REPRODUCTIVE HEALTH RESEARCH UNIT	INJECTABLE AND ORAL CONTRACEPTIVE BONE MASS STUDY ADMISSION QUESTIONNAIRE	ADM Page 3
--	--	-----------------------

Subject study number

--	--	--

19. We would like to know what methods of contraception you have used in the last five years?

ENTER METHOD CODE FROM THE LIST BELOW IN THE CALENDAR BELOW

A = ABORTION / MISCARRIAGE
B = DELIVERY
C = BREAST FEEDING
D = PREGNANCY
E = DEPO
F = NURISTERATE
G = COMBINED ORAL PILLS
H = PROGESTOGEN-ONLY PILLS

J = FEMALE STERILIZATION
K = VASECTOMY
L = MALE / FEMALE CONDOMS
M = IUD
N = NATURAL FAMILY PLANNING SAFE PERIOD
O = RHYTHM METHOD, WITHDRAWAL
P = SEX WITH NO CONTRACEPTIVE METHOD
Q = ABSTINENCY
R = OTHER, SPECIFY _____

(If two or more methods were used during the name period, please mark all the methods)

(if two or more methods were used during the time period, please mark all the methods)

YEAR	AGE AT 1 JAN	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	NOTES
2002														
2001														
2000														
1999														
1998														
1997														
1996														

19. Summary of duration of contraceptive use

	AGE AT FIRST USE		TOTAL DURATION DURING LAST 5 YEARS		TOTAL LIFETIME DURATION			
Method	Years		Years	Months		Years Months		
COMBINED ORAL CONTRACEPTIVES								
PROGESTOGEN-ONLY PILLS								
DMPA								
NURISTERATE								
IUD								
CONDOM								
FEMALE STERILIZATION								
VASECTOMY								
NATURAL FAMILY PLANNING								

REPRODUCTIVE HEALTH RESEARCH UNIT	INJECTABLE AND ORAL CONTRACEPTIVE BONE MASS STUDY ADMISSION QUESTIONNAIRE	ADM Page 4
--	--	-----------------------

Subject study number

We would like to ask you some questions about smoking, alcohol and caffeine.

19. Have you ever smoked tobacco / cigarettes?

☐

NO.....1-----Q25

YES.....2-----Q22

Are you a current smoker?

☐

NO.....1-----Q24

YES.....2-----Q23

20. What is the average number of cigarettes you have
Smoked per day in the last month?

NUMBER PER DAY

☐

If no longer smoking now, at what age did
You stop?

YEARS

☐

21. How many cups / bottles of the following beverages

Do you drink in a typical week: (code 00 if none)

a) Coffee with caffeine cups

b) Decaffeinated coffee cups

c) Tea cups

d) Rooibos cups

Fizzy drinks 250 – 340ml

e) Cola/ bottles / cans

f) Other fizzy drinks bottles / cans

BRANDS _____

Alcoholic drinks

g) Beer bottles / cans

h) Wine glasses

i) Spirits 25 ml shots

j) Cider bottles/cans

26. Have you dieted in the last year

No.....1

Yes.....2

☐

27. Do you exercise regularly at least once a
week

No.....1

Yes.....2

☐

28. FSH

29. LH:

Study group:

☐

Interviewers Name: _____

Remarks :

REPRODUCTIVE HEALTH RESEARCH UNIT	INJECTABLE AND ORAL CONTRACEPTIVE BONE MASS STUDY ADMISSION QUESTIONNAIRE	ADM Extra page
--	--	---------------------------

Subject study number

Children	Month and Year of birth	Total breast feeding (months)
7 th child		
8 th child		
9 th child		
10 th child		

REPRODUCTIVE HEALTH RESEARCH UNIT	INJECTABLE AND ORAL CONTRACEPTIVE BONE MASS STUDY EXAMINATION FORM	EXA Page.1
--	---	-----------------------

Subject study number Visit code Date of examination
Day Month Year

1. Height CM .
 2. Weight KG .

3. Blood pressure Systolic (mmHg)
 Diastolic (mmHg)

4. Bone mass measurement

Previous Fracture L or R ☐

No.....1
 Yes.....2

1. RADIUS BMC .
 BMD .
 AREA .

2. ULNA BMC .
 BMD .
 AREA .

3. DISTAL BMC .
 BMD .
 AREA .

5. Arm circumference (midpoint of upper arm) CM .

6. Waist circumference CM .

7. Hip circumference CM .

8. Blood drawn for FSH measurement ☐

NO.....1
 YES.....2

9. Any evidence of disease in the following systems YES NO

a) Cardiovascular Specify ----- ☐ ☐

b) Respiratory Specify ----- ☐ ☐

c) Hepatic Specify ----- ☐ ☐

d) Renal Specify ----- ☐ ☐

e) Gastrointestinal Specify ----- ☐ ☐

f) Neurological Specify ----- ☐ ☐

g) Endocrinological Specify ----- ☐ ☐

Remarks:

Interviewers name

Signature: -----

REPRODUCTIVE HEALTH RESEARCH UNIT	INJECTABLE AND ORAL CONTRACEPTIVE BONE MASS STUDY SIX MONTHLY FOLLOW-UP QUESTIONNAIRE	FOL Page 1
--	--	-----------------------

Subject study number				Study group	☐	Date of interview						
Visit number						Day	Month	Year				

1a) Are you currently using a method of contraception? ☐

- NONE.....0
 DEPO.....1
 NURISTERATE.....2
 COMBINED ORAL PILLS.....3-----1b
 PROGESTOGEN-ONLY PILLS.....4-----1b
 IUD.....5
 FEMALE STERILIZATION.....6
 BARRIER.....7
 NATURAL METHODS.....8
 MALE STERILIZATION.....9
 OTHER.....99

1b) Brand of pill ----- ☐

2. Has contraceptive method changed, stopped or started since last visit? ☐

- NO.....1-----Q5
 YES.....2

3. When did you change method?

MONTH

YEAR

4. What did you change from ----- (method) ☐

- NONE.....0
 DEPO.....1
 NURISTERATE.....2
 COMBINED ORAL PILLS.....3
 PROGESTOGEN-ONLY PILLS.....4
 IUD.....5
 FEMALE STERILIZATION.....6
 BARRIER.....7
 NATURAL METHODS.....8
 MALE STERILIZATION.....9
 OTHER.....99

5. Since we last saw you have you Experienced any of the following? YES NO

- a) Hot flushes ☐ ☐
- b) Night sweats ☐ ☐
- c) Skin changes ☐ ☐
- d) Dry vagina ☐ ☐

6. Has subject had any of the following since their last visit? YES NO

- a) Weight loss (5 kg or more in the last six months) ☐ ☐
- b) Chronic diarrhoea (in the last six months) ☐ ☐
- c) Lymphadenopathy ☐ ☐
- D) Tuberculosis ☐ ☐

We would like to ask you about any changes in your, smoking, alcohol , tea and coffee drinking since we last saw you.

7. Are you a current smoker? ☐

NO.....1..... Q10

YES.....2.....Q8

8. What is the average number of cigarettes you smoked per day in the last month ?

NUMBER PER DAY

9. If no longer smoking, at what age did you stop?

YEARS

REPRODUCTIVE HEALTH RESEARCH UNIT	INJECTABLE AND ORAL CONTRACEPTIVE BONE MASS STUDY SIX MONTHLY FOLLOW-UP QUESTIONNAIRE	FOL Page 2
--	--	-----------------------

Subject study number

Visit number

10. How many cups/bottles of the following beverages

a). Coffee with caffeine cups

b). Decaffeinated coffee cups

c). Tea cups

d). Rooibos tea cups

FIZZY DRINKS 250-340ml

e). Cola bottles/cans

f). Other fizzy drinks bottles/cans

BRANDS _____

Alcoholic drinks

g). Beer bottles/cans

h). Cider bottles/cans

i). Wines bottles/cans

j). Spirits 25ml shots

11. Have you dieted in the last six months? ☐

NO.....1
YES.....2

12. Do you exercise regularly atleast once a week? ☐

NO.....1
YES.....2

13a) Do you have a long standing illness? ☐

NO.....1
YES.....2

13b). If yes, can you tell us what this illness is?

LIST DISEASE

ICD 10

14a). Are you currently taking any long term medication?

☐

NO.....1
YES.....2-----Part b

14b) If yes, what medication are you taking?

LIST -----

15.) Are you pregnant? ☐

NO.....1
YES.....2

16. What is your last date of menstruation? Day Month Year

16b) Date of last injection Day Month Year

17. Previous fracture L or R ☐

NO.....1
YES.....2

18. FSH

19. LH

Remarks:

Interviewers name: -----

Signature: -----

--

REPRODCUTIVE HEALTH RESEARCH UNIT	INJECTABLE AND ORAL CONTRACEPTIVE BONE MASS STUDY DISCONTINUATION FORM	DIS Page 1.
--	---	------------------------

Subject study number

Study group

Visit number

1. Date of follow-up terminated

Day Month Year

2. (a) Reason for termination

1 = end of study

2 = moved out of area

3 = death

4 = personal

5 = other reason

☐

(b) If "other reason", or personal specify

CODE

3. If "DEATH",

(a) Date of death

Day Month Year

(b) Cause of death

(i) _____

 .

(ii) _____

 .

(iii) _____

ICD code

Remarks and additional information:

Name of investigator: _____

Signature: _____

REPRODUCTIVE HEALTH RESEARCH UNIT	INJECTABLE AND ORAL CONTRACEPTIVE BONE MASS STUDY FOLLOW-UP (NON-ATTENDANCE)	NON PAGE 1.
--	---	----------------------------------

Subject study number

Study group

Visit number

1. Date of follow-up

Day Month Year

2. Follow-up number

3. Mode of follow-up 1 = telephone
2 = home visit

4. Subject contacted 1 = No
2 = Yes

If yes, what was the outcome?

1 = discontinued (complete discontinuation form)
2 = arranged new appointment

If no, indicate in remarks section action to be taken

Remarks and additional information:

Name of investigator: _____

Signature: _____

Explanatory notes on the questionnaires

A. General Principle

All blank boxes on the Forms must be filled in except:

- (i) When the form permits you to skip questions, or part of questions, indicated by an “IF, GO TO Q” instruction;

When the question contains an “IF, LEAVE BLANK” instruction.

When the number of digits in the code being entered is less than the number of boxes provided, a leading zero (0) should be put in the remaining boxes. For example, in the case of a subject, who has had 2 pregnancies, the coding should be 02, Q14 of admission form.

Please note that no digits after the decimal point should be entered, except where a decimal point is printed on the form (e.g. for recording of waist and hip in Q7 and 8 of examination form). When numbers need to be rounded off, 5 or greater should be rounded off upwards, 4 or less downwards.

When filling out the forms, interviewers should preferably first circle the correct answer from the list of options provided, and then enter the appropriate code in the boxes. This will facilitate cross checking of the codes.

If the answer to a question is not known, code 9, 99, 999, etc. should be used depending on the number of boxes provided. The same applies also when part of the answer is unknown, e.g., if only the year of birth is known (1958), Q1 of the Screening Form should be coded 9958. Although the use of 9, 99, 999, etc. codes for “don’t knows” is not likely to lead to confusion

with actual numbers, interviewers are requested to also write down on the forms “don’t know” when using these codes.

Corrections to forms

Any information recorded inaccurately should be crossed out and correct answer written in red pen next to the incorrect. The correction should be initiated by the member of staff who made the change. Any information added to the questionnaires at a later date e.g. coding COD codes, should be written in green pen to indicate added information.

B. Notes about the individual forms

SCREENING QUESTIONNAIRE (SCR)

These forms were used to screen potential candidates for the study. The Screening Form contains, in questionnaire form, the selection criteria for participation and ensures that only those women were recruited, who fulfill all criteria. All completed screening forms were kept, including those of women not enrolled in the study. This provided information on the number of women screened and the number eventually recruited.

ADMISSION QUESTIONNAIRE (ADM)

This form was completed at the time of admission. The subject number in the form should be filled in by the interviewer.

For the main phase of the study, subject numbers **should be used sequentially**, starting from 001, without taking in to consideration the serial numbers used for the pilot phase.

QUESTIONS THAT MERIT SOME COMMENT ARE:

Q1. (Age at last birthday)

Enter the age in completed years at her last birthday.

Q3a and b (Highest level of education)

Enter either highest grade or standard passed. If she has further qualifications enter highest qualification passed in part b.

Q4 (Main Occupation)

Type of occupation not required only classify type of occupation listed.

Student is classified as someone pursuing further qualifications i.e. has completed schooling.

Q5 Income – This should be total household income and not just income of subject

If not known put in 9999

Q6 Relationship status.

If a women is divorced or widowed and has no current partner enter option “5” single (no partner). If she now has a new partner use option 1-4 to classify her current relationship.

Q10 Do you menstruate regularly?

Regular menstruation is defined as periodic cyclical bleeding occurring at intervals of between 21-35 days.

Q16 (Breast-feeding)

The questions should be as asked only for children breast-fed. Space in the form has been provided for 6 children. If more than 6 children were breast-fed, information may be provided on a separate sheet.

(a) Record date the child was born

(b) Enter number of months, the child was either exclusively or partially fed breast milk (supplementation).

Q17 Which contraceptive method are you currently using?

For women in the 15-19 age group this question will act as a check to screening forms. For women in this age group who accept a hormonal method on that day the response should be either coded as None or any other non-hormonal method in the list (e.g. barrier or natural method). The hormonal method started on that day will be recorded on the screening form Q10 & Q19 of the Admission form.

Q18 Name of the Pill

Check list provided on form. Remember that POP users are not included.

Q19 (History of contraceptive use in the last five years)

These questions relate to past and present use of contraception. Since contraceptive use may be interrupted by events like pregnancy or personal reasons, space has been provided to enter details for each episode of the method used.

15 – 19 age group:- Probe for hormonal method use in the past that may not have been indicated in screening. Record any method used such as condoms or sex with no method.

Q20 Assist the woman in identifying the method that she used in the past.

Study Group: The study involves recruitment of subjects in 8 groups. Following codes should be used to indicate the group, the subject has been assigned to:

Code A - 15-19 new user DMPA

- B - 15-19 new user NET-EN
- C - 15-19 non-user hormonal contraceptives
- D - 15-19 new user COC's
- E - 40-49 DMPA use
- F - 40-49 NET-EN user
- G - 40-49 non-user
- H - 40-49 COC user

Q25 Beverages

It may be difficult for the women to be exact about the number of can or bottles of these drinks she drinks in a week. Probe for regular consumption and if only beverage is mentioned not on the list, put brand in the other section and this will be investigated and recorded at a later date. Bottle / can quantity relate to those between 250–340 ml. Ensure that the subject is aware of this size. If larger bottles are mentioned (1-2l L) revise entry i.e. 1 litre bottle = 3 cans (340ml) or 4 bottles (250ml).

Q27 Regular exercise

For purposes of this study exercise is defined as a distinct activity i.e. aerobics, jogging etc.

EXAMINATION QUESTIONNAIRE (EXA)

To avoid interobserver variation: One person from the study was responsible for conducting the examination, especially anthropometry (height, weight, arm/waist/hip circumference). In the event of leave or sickness, the principal investigator or another trained member of staff conducted the examination.

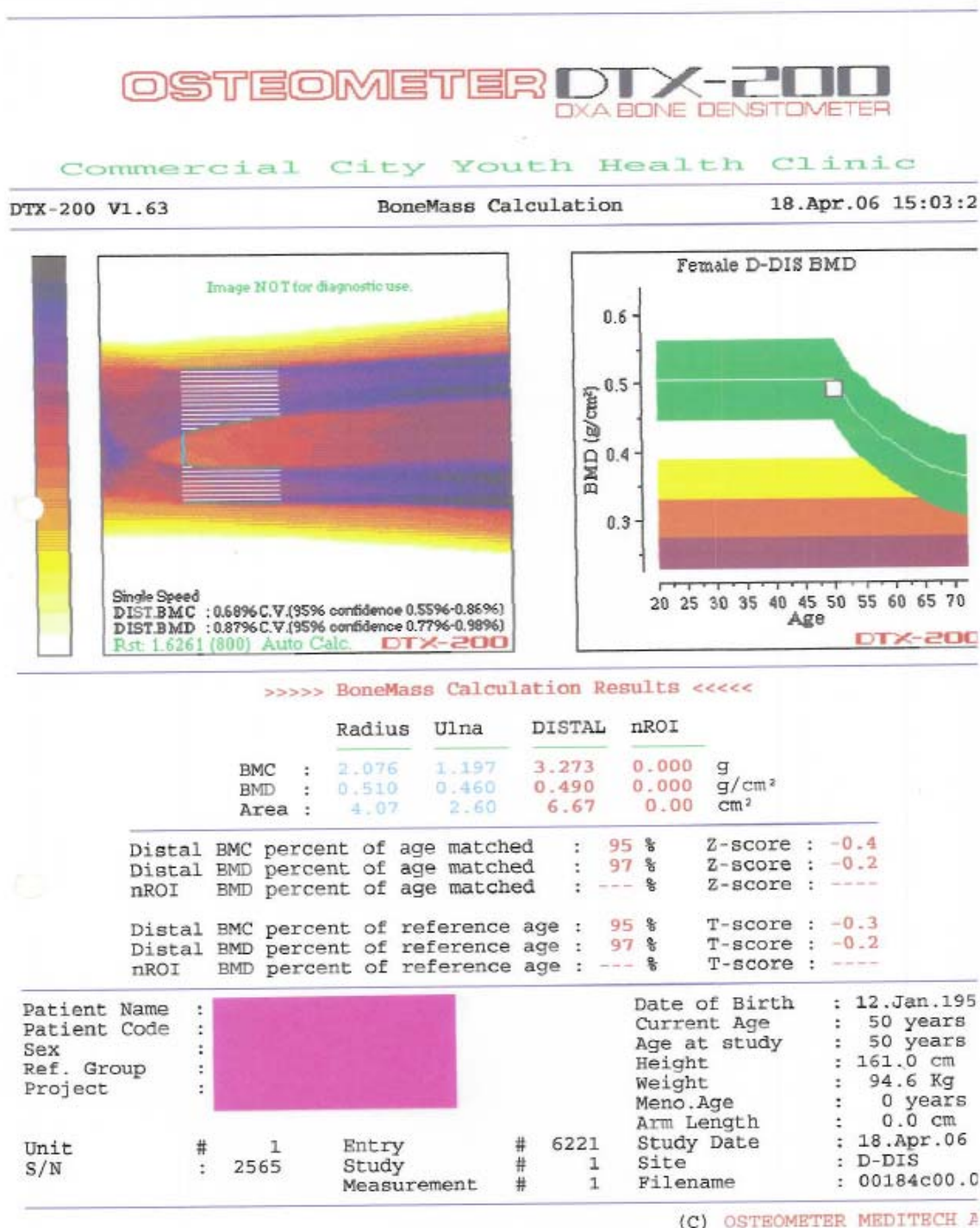
FOLLOW-UP QUESTIONNAIRE (FOL)

Q2. Check original group and last visit to validate information given by subject.

Q6.a Weight loss to be cross-checked with past visit examination form.

APPENDIX 3

INFORMATION ON DEXA EQUIPMENT



OSTEOMETER DTX-200

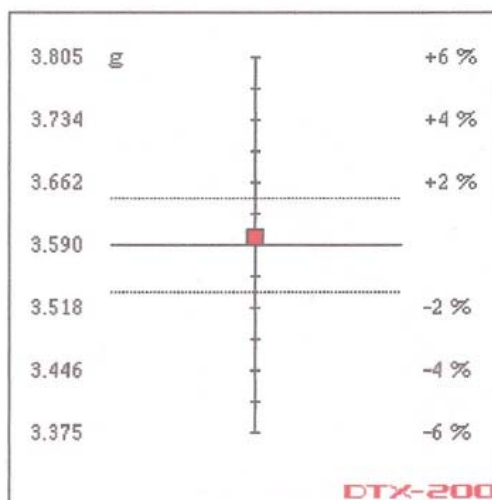
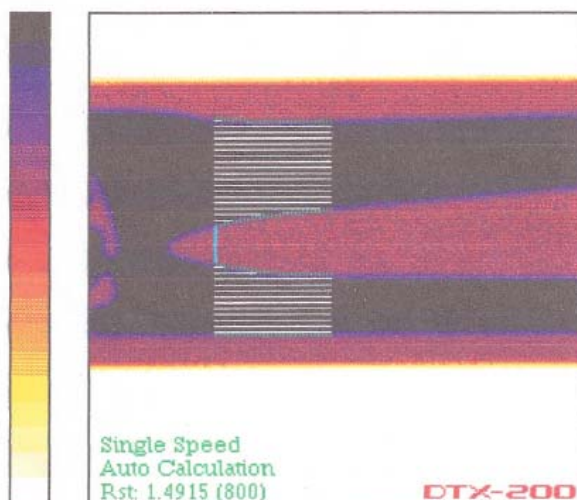
DXA BONE DENSITOMETER

Commercial City Youth Health Clinic

DTX-200 V1.63

Phantom Measurement

07.Jun.04 8:48:05



>>>> Phantom Measurement Results <<<<

Distal BMC : 3.597 g
Distal BMD : 0.391 g/cm²
Distal Area : 9.20 cm²

Deviation from Expected : 0.2 %

Unit	#	S/N
	1	2565

(C) OSTEOMETER MEDITECH A/S

OSTEOMETER DTX-200

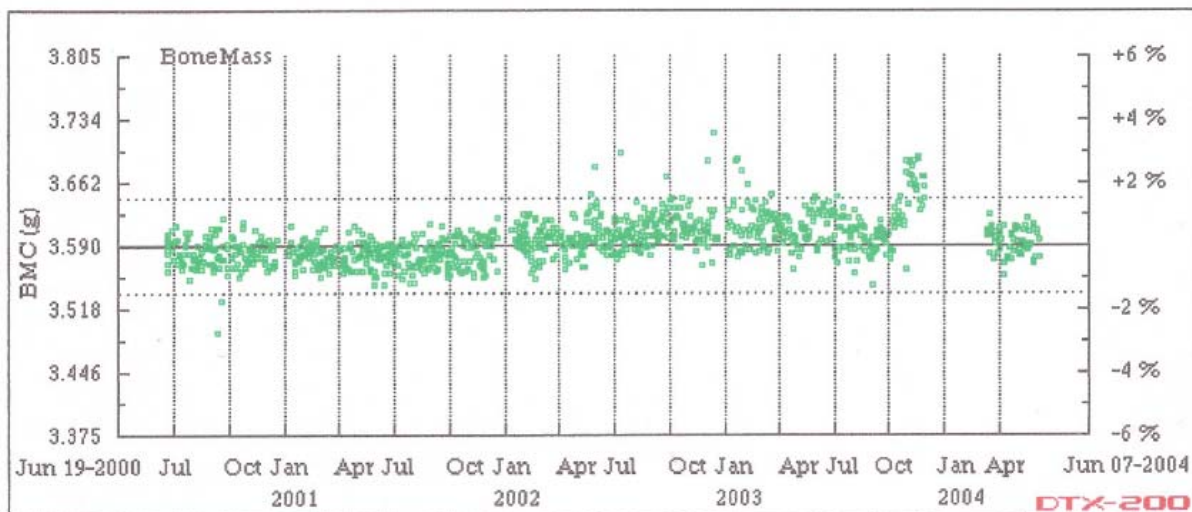
DXA BONE DENSITOMETER

Commercial City Youth Health Clinic

DTX-200 V1.63

Phantom Statistics

07.Jun.04 14:50:41



>>>> Phantom Statistics Results <<<<

Mean Distal BMC : 3.596 g
 Mean Distal BMD : 0.389 g/cm²
 Mean Distal Area : 9.240 cm²
 BMC Variation : 0.71 %
 BMD Variation : 0.53 %
 Area Variation : 0.58 %

Number of Measurements : 1003
 Selected Period : 1449 days

Unit : # 1
 S/N : 2565

DRS JACKPERSAD, & PARTNERS INC.
WESTRIDGE MEDICAL CENTRE
X-RAY DEPARTMENT

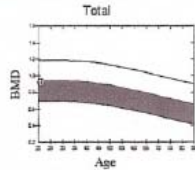
Phone: (031)273-1050

Fax: (031)273-1055

Name:		Sex:	Female	Height:	154.0 cm
Patient ID:		Ethnicity:		Weight:	64.6 kg
Age:	21	Date of Birth:	09/27/1984		



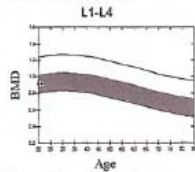
Scan Date: 03 February 2006
Scan ID: A0203060I



Scan Type: x Left Hip

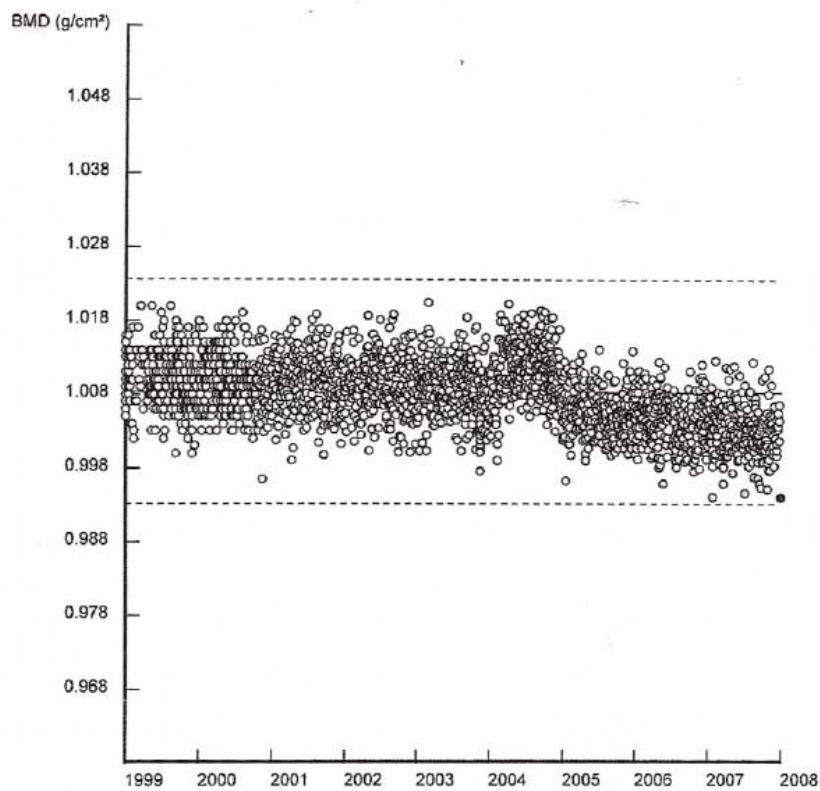


Scan Date: 03 February 2006
Scan ID: A0203060J



Scan Type: x Lumbar Spine

Summary:



Setup	Reference Values	Plot Statistics
a Lumbar Spine phantom #7805 System S/N: 49369	Limits: $\pm 1.5\%$ of mean Mean: 1.008 (g/cm ²) SD: 0.039 (g/cm ²)	Number of Points: 2509 Mean: 1.008 (g/cm ²) SD: 0.004 (g/cm ²) CV: 0.438 %

2. INTRODUCTION

2.1 DTX-200 Technology

The Osteometer DTX-200 estimates the bone mineral content (BMC, grams) and bone mineral density (BMD, grams/cm²) in the distal section of the forearm (radius and ulna).

Technology:

The DTX-200 uses the technique of X-ray absorptiometry. The X-ray tube is supplied with a high voltage of 55 Kv. The beam is filtered with tin (Sn), yielding two effective energy levels for separation of bone and soft tissue (fat, muscle, etc).

How the DTX-200 works:

The forearm is placed in a container during the examination. The X-ray source is located on one side of the forearm and a detector is located on the other side. During an examination, the source and the detector move synchronously in a number of scan paths across the distal forearm. The mechanical set-up provides an automatic line by line internal reference calibration during scanning in order to achieve drift-free measurements. No calibration is necessary by the operator. The X-ray signal registered at the detector is inversely proportional to the bone mineral content of the forearm. When more bone is present, fewer X-rays are detected. When the bone mass is low, more X-rays are detected. The bone mass results are automatically calculated.

Quality control (QC):

The quality control (QC) is a procedure for verifying that the DTX-200 is operating properly. This procedure must be carried out each day before the first patient is scanned. Refer to chapter 6 where the quality control is fully described.

NOTE: The DTX-200 automatically corrects for amounts of fat mass in the forearm.

NOTE: If the patient is under adequate estrogen therapy there is no current evidence that serial bone mass measurements need to be performed.

2.2 Precision and Accuracy

Precision:

The precision is the ability to scan a patient or a phantom and obtain the same result each time. The In Vivo precision of DTX-200 measurements were estimated by measuring the distal area of the non-dominant forearm in 15 volunteers 5 times each with repositioning between all replicate measurements. The In Vivo precision is not only dependent on the intrinsic machine precision of the equipment, but also on the cooperativeness of the subject (ability to remain still) and the positioning techniques.

The In Vivo Precision for the above measurements:

Distal	BMC	0.68 %
Distal	BMD	0.87%
n.ROI	BMD	1.11%

Serial patient measurements should be performed on the same service.

Accuracy:

The accuracy is defined as the degree of conformity of a measure to standard or true value.

To assess the accuracy of the DTX-200 Bone Densitometer, repeated findings using a phantom have been done. The accuracy for the DTX-200 Bone Densitometer is within $\pm 0.5\%$ when measuring the bone mineral content of an Osteometer forearm phantom.

APPENDIX 4

Ethics Approval Certification (University of the Witwatersrand)

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

COMMITTEE FOR RESEARCH ON HUMAN SUBJECTS (MEDICAL)

Ref: R14/49 Beksinka

CLEARANCE CERTIFICATE

PROTOCOL NUMBER M981001

PROJECT

Bome Density & Use Of Injectable Progesterone
Only Contraceptives: Depot Medroxy-
progesteron Acetate (DMPA), Norethisterone
Enanthate (NET-EN) & Combined Oral
Contraceptives

INVESTIGATORS

Ms M Beksinka

DEPARTMENT

Reproductive Health, Baragwanath Hospital

DATE CONSIDERED

981030

DECISION OF THE COMMITTEE *

Approved unconditionally

DATE 981111

CHAIRMAN.....



(Professor P E Cleaton-Jones)

* Guidelines for written "informed consent" attached where applicable.

c c Supervisor: Ms M Beksinka

Dept of Reproductive Health, Baragwanath Hospital

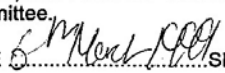
Works2Uain0015HumEth97.wdbIM 981001

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10001, 10th Floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee.

DATE.....



SIGNATURE.....



PROTOCOL NO.: M 981001

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Prof Cleaton Jones
Chairman
Human subjects Ethics Committee
Wits University

2 nd September 05

Dear Prof Cleaton Jones

RE: protocol number 981001:Bone Density and use of Progestogen-only injectable Contraceptives or Combined oral contraceptives, Durban.

The above study has now entered its 6th and final year of follow-up. Bone mineral density is currently measured at a forearm site. There is some concern that this site is not as sensitive to the effects of progestogen only contraception. The WHO who fund the study have therefore requested that the study measures the hip and spine of a subsample of participants (approx n=150). We do not have the equipment to do this however in our office building there are a group of specialist diagnostic radiologists (Jackpersad & partners inc)where this measurement could be done. This would be a one off measurement and would require an additional visit by the participant. The study nurse would be present and the scan will take about 1 minute to conduct. The equipment is a Hologic QDR4500 and poses no risk to the participant. This method is safe and uses low radiation DEXA (dual-energy X-ray absorptiometry. We have drafted an additional consent form for the participant (attached) and a letter explaining the procedure and asking them if they wish to volunteer. The study nurse will explain what is involved and will book an appointment for the participant at a time convenient for them and will provide a map. The existing study site is about 1 km away from the medical centre.

We request that the ethics committee considers the documents supplied and grants us an ammendment to the existing protocol.

Kind regards
Mags Beksinska
Director Durban
Reproductive Health and HIV Research Unit

APPENDIX 5

FLYERS AND POSTERS USED IN RECRUITMENT

CONTRACEPTIVE STUDY

AGE GROUP 15-19

The Reproductive Health Research Unit is undertaking a study on the effect of contraceptives on the bone mass (bones of the skeleton).

*The study would like to involve women participants who are:
Between 15 and 19 years, new acceptors of injectables, oral contraceptives and condoms.*

The Research Nurse will conduct a short interview on general health related issues. She will also measure your bone mass which involves one of your forearms. Your height, weight and blood pressure will be part of the routine check. The whole procedure, including interview and bone mass measurements will take $\pm 20 - 25$ minutes of your time.

The transport cost will be covered by the study.

**For more information on the study, contact the nurses:
Family Planning Clinic, Commercial Road 031 307 2781**

CONTRACEPTIVE STUDY

AGE GROUP 40-49

The Reproductive Health Research Unit is undertaking a study on the effect of contraceptives on the bone mass (bones of the skeleton).

*The study would like to involve women participants who are:
Between 40 and 49 years, on injectables, oral contraceptives, condoms, loop and those who are sterilized.*

The Research Nurse will conduct a short interview on general health related issues. She will also measure your bone mass which involves one of your forearms. Your height, weight and blood pressure will be part of the routine check. A blood sample will be taken to check if you are menopausal. The whole procedure, including interview and bone mass measurements will take $\pm 25 - 30$ minutes of your time.

The transport cost will be covered by the study.

**For more information on the study, contact the nurses:
Family Planning Clinic, commercial Road 031 307-2781**

APPENDIX 6

REFERRAL LETTERS

Referral letter for General Practitioner

Age 40-49

Doctor

Dear Doctor

RE: Bone Density and use of Depot Medroxyprogesterone Acetate (DMPA), Norethisterone Enanthate (NET-EN) and Combined Oral Contraceptives

Your patient Ms.....has been involved in the above study at the Commercial City Family Planning Clinic, Commercial Road, Durban. The study measurements include a bone mass scan of the forearm, height, weight, blood pressure, waist / hip / forearm measurement and blood test. The study has found that:-

(Reason for referral)

We attach details on the findings for you. Please contact us at the above if you require any further information.

Yours sincerely

M. Beksinska
Provincial Director

Referral letter for General Practitioner

Age 15 - 19

Doctor

Dear Doctor

RE: Bone Density and use of Depot Medroxyprogesterone Acetate (DMPA), Norethisterone Enanthate (NET-EN) and Combined Oral Contraceptives

Your patient Ms.....has been involved in the above study at the Commercial City Family Planning Clinic, Commercial Road, Durban. The study measurements include a bone mass scan of the forearm, height, weight, blood pressure, and waist / hip / forearm measurement. The study has found that:-

(Reason for referral)

We attach details on the findings for you. Please contact us at the above if you require any further information.

Yours sincerely

M. Beksinska
Provincial Director

COLLABORATORS

1. World Health Organisation

1211 Geneva 27

Switzerland

Contact: Dr Tim Farley

Nature of collaboration:

- Technical support and advice
- Funding

2. Professor J Moodaley / Dr Maharaj / Dr M Popis

Dept of Obstetrics & Gynaecology

Medical School

King Edward Hospital

Umbilo Road

Durban

Nature of collaboration:

- Referral
- Technical support and advice
- General interest in study

3. Dr Trudie Machattie

Addington Hospital

Dept of Chemical Pathology

Prince Street

Durban

Nature of collaboration:

- Analysis of blood samples

4. Carol McIntosh

Addington Hospital

Dept of Chemical Pathology

Prince Street

Durban

Nature of collaboration:

- Processing and storage of blood samples

5. Mrs Krystal Kaiser
Dept of Health KwaZulu Natal

Nature of collaboration:

- Participation of clinic and provision of consulting space for study
- Clinic staff support of study

6. Mark Roulliard
4001-4006 Juniper House
92 Overport Drive
Overport
Durban
4001
Telephone: 031-201 1321

Nature of collaboration

- Maintenance and servicing of equipment

APPENDIX 8

Publications in Peer reviewed Journals

Beksinska M.E., Kleinschmidt I., Smit J.A., Farley T.M.M. 2009. Bone Mineral Density in a cohort of adolescents during use of norethisterone enanthate, depot-medroxyprogesterone acetate or combined oral contraceptives and after discontinuation of norethisterone enanthate. *Contraception*, vol. 79, no.5 pp.345-349.

Beksinska ME, Kleinschmidt I, Smit JA, Farley TMM, Rees H. 2009. Bone mineral density in young women aged 19-24 after 4-5 years of exclusive and mixed use of hormonal contraception. *Contraception*, vol.79 no.8 August [In press].

Beksinska M, Kleinschmidt I, Smit J, Farley MM. 2007. Bone mineral density in adolescents using norethisterone enanthate, depot-medroxyprogesterone acetate, or combined oral contraceptives for contraception. *Contraception*, vol.75 no.6, pp.438-43.

Beksinska M, Smit J, Kleinschmidt I, Farley T, Mbatha F. 2005. Bone mineral density in women aged 40-49 years using depot-medroxyprogesterone acetate, norethisterone enanthate or combined oral contraceptives for contraception. *Contraception*, vol.71, no. 3, pp.170-5.

Beksinska ME, Smit J, Kleinschmidt I, Rees H, Farley TMM Guidozi F. 2003 Detection of raised FSH levels among older women using depot medroxyprogesterone acetate and norethisterone enanthate. *Contraception*, vol. 68, no. pp.339-43.

Original research article

Bone mineral density in a cohort of adolescents during use of norethisterone enanthate, depot-medroxyprogesterone acetate or combined oral contraceptives and after discontinuation of norethisterone enanthate[☆]

Mags E. Beksinska^{a,*}, Immo Kleinschmidt^b, Jenni A. Smit^a, Timothy M.M. Farley^c

^aReproductive Health and HIV Research Unit, Department of Obstetrics and Gynaecology, University of the Witwatersrand, Mayville 4091, South Africa

^bLondon School of Hygiene and Tropical Medicine, WC1E London, UK

^cUNDP/UNFPA/WHO/World Bank Special Programme of Research, Development and Research Training in Human Reproduction, Department of Reproductive Health and Research, World Health Organization, CH 1211 Geneva 27, Switzerland

Received 31 October 2008; revised 11 November 2008; accepted 17 November 2008

Abstract

Background: Depot medroxyprogesterone acetate (DMPA), norethisterone enanthate (NET-EN) and combined oral contraceptives (COCs) have been shown to have a negative effect on bone mineral density (BMD) in adolescents. The aim of this study was to investigate BMD in 15- to 19-year-old new users of DMPA, NET-EN and COCs.

Study Design: This 5-year longitudinal study followed up new users of DMPA ($n=115$), NET-EN ($n=115$) and COCs ($n=116$) and 144 nonuser controls. BMD was measured at the distal radius using dual-energy X-ray absorptiometry.

Results: BMD increased in all groups (annual percent increase: nonusers, 1.49%; DMPA, 1.39%; NET-EN, 1.03%; COCs, 0.84%) during follow-up ($p<.001$). There was evidence for lower BMD increases per annum in NET-EN ($p=.050$) and COC ($p=.010$) users compared to nonusers but no difference between DMPA and nonusers ($p=.76$). In 14 NET-EN discontinuers, an overall reduction of 0.61% per year BMD was followed upon cessation by an increase of 0.69% per year ($p=.066$).

Conclusion: This study suggests that BMD increases in adolescents may be less in NET-EN and COC users; however, recovery of BMD in NET-EN users was found in the small sample of adolescents followed post-discontinuation.

© 2009 Elsevier Inc. All rights reserved.

Keywords: Bone mineral density; DMPA; NET-EN; COCs; Adolescents

1. Introduction

Depot medroxyprogesterone acetate (DMPA) has been found to have a negative effect on bone mineral density (BMD) in adult premenopausal women [1–5] and in adolescents [6–13]. Limited data on the effect of norethisterone enanthate (NET-EN) have found a negative effect on BMD in adult [14] and adolescent users [15]. A comprehensive review concluded that combined oral contraceptive (COC) use in adult premenopausal women was not associated with changes in BMD [16]. However, emerging

data show that BMD may be compromised in adolescent users of low-dose COCs [10,17,18].

Studies that have followed women after discontinuation of DMPA have found that BMD recovers in adult premenopausal women within approximately 2–3 years of cessation of the method [19,20]. Two studies have followed up adolescent users of DMPA post-discontinuation [12,13], and in both these studies, a significant increase in BMD was found in adolescents who discontinued DMPA. No longitudinal data are available on recovery of BMD in adolescent NET-EN and low-dose COC users. More evidence is needed to show if the recovery of BMD found in adult users of hormonal contraception is replicated in adolescents. The objective of this study was to determine if long-term use of hormonal contraceptives (COC, DMPA and NET-EN), compared to nonuse, was associated with a change in bone mass in women aged 15–19 years.

[☆] This study was supported by a grant from the World Health Organization, The William and Flora Hewlett Foundation and The Department for International Development UK (DFID).

* Corresponding author. Tel.: +27 31 261 8840; fax: +27 31 261 8868.
E-mail address: mbeksinska@rhu.co.za (M.E. Beksinska).

2. Subjects and methods

This was a prospective longitudinal study of adolescents aged 15 to 19 years who had never used hormonal contraception prior to recruitment to the study. Initiators of DMPA, NET-EN, and COCs and nonusers of contraception were enrolled from a family planning clinic in Durban, South Africa. The study cohort was recruited between July 2000 and July 2002 and follow-up continued until April 2006. All DMPA users were started on a regimen of 150 mg every 12 weeks, and NET-EN users were started on a regimen of 200 mg every 8 weeks. Both DMPA and NET-EN were administered intramuscularly. The COCs used by women included a range of formulations, with almost all (93%) using low-dose formulations containing between 30 and 40 mcg of estrogen. Women were eligible to participate if they had not lactated or delivered in the past 6 months, were not currently or had never used medication known to affect calcium metabolism for more than 3 months and did not have a chronic disease affecting calcium metabolism. At the baseline visit, participants' height, weight and blood pressure were measured using a standard protocol and a questionnaire was administered to elicit information on demographic characteristics, regularity of the menstrual cycle, smoking, diet, exercise and caffeine and alcohol intake.

Forearm BMD was measured by dual-energy X-ray absorptiometry (DEXA model DTX-200, Osteometer Mediatech A/S Co., Rodovre, Denmark). BMD was measured in grams per square centimeter at the distal radius in the forearm. The DEXA equipment was standardized daily using a phantom as prescribed by the manufacturer's instructions. Accuracy to the standard during the recruitment and follow-up period was 0.53%.

Study participants were followed up at approximately six monthly intervals. Forearm BMD, height and weight were measured at each follow-up visit. History of contraceptive use since last visit was recorded. Those recruited between July 2000 and April 2001 and who continued to participate until the end of the study completed 5 years of follow-up. Those recruited after April 2001 who continued until the end of the study completed between 4 and 5 years of follow-up. Follow-up could not continue beyond April 2006 when project funding came to an end. Women continued the same follow-up schedule even if they stopped, changed or started another contraceptive method in order to investigate issues of recovery of BMD post-discontinuation of a hormonal method.

The characteristics of women in the study were quantified as means $\pm$ SD, medians or percentages. The study was powered to detect a half SD difference in bone mass between users and nonusers of hormonal contraceptives. This would be of biological significance as this difference is expected to translate into a large difference in the risk of fracture in older women. Information on the mean and SD of cross-sectional measurements of forearm bone mass in White, European, premenopausal women reported by Nordin [21] was used to

estimate sample size. A sample of 63 participants per contraceptive group was required, assuming a two-tailed statistical test with a significance level of 5% and a power of 80%. Allowing for 10–15% loss to follow-up per year, this sample was increased to at least 110 women in each group to ensure adequate numbers in long-term follow-up. The nonuser group was overrecruited compared to other groups as, in addition to loss to follow-up, it was anticipated that some of these women would commence a method of contraception or become pregnant.

Differences in BMD between contraceptive groups and the associations between BMD and selected characteristics of the study participants by contraceptive group were assessed using one-way analysis of variance and multiple variable linear regression. BMD was measured in users and discontinuers.

Follow-up of all women was cumulated up to their last visit or up to the visit preceding a visit at which they reported a change in contraceptive method, whichever occurred first.

Interim analysis of this study found that BMI was significantly associated with radius BMD [15]. To assess the effect of contraceptive method on radius BMD and to allow for within-subject correlation of responses while controlling for the confounding effect of changes in BMI, we used random-effects linear regression methods with radius BMD at the time of a study visit as the response variable and contraceptive method, duration of follow-up and BMI at time of the visit as explanatory variables (STATA V.10, College Station, TX, USA). Differences in rate of change in radius BMD per year between methods were calculated by including interaction terms in the model for method and follow-up time. Additionally, we investigated whether change in radius BMD varied between two time periods by including an interaction term between follow-up time up to the last visit occurring before 2.5 years and follow-up time between 2.5 and 5 years, for each user group separately. These two time periods were chosen as the literature indicates that the greatest loss of BMD as a result of hormonal contraceptive use occurs over the first 2 years of use [22] and then loss continues at a slower rate and appears to stabilize. Finally, we investigated BMD in women who discontinued hormonal contraception.

Ethical approval was granted by the University of the Witwatersrand, Human Subjects Research Committee (Protocol Number M981001), and by the Scientific and Ethical Review Group of the World Health Organization (WHO).

3. Results

In total, 490 women aged 15–19 years were recruited. All women including those in the DMPA, NET-EN and COC groups had no past use of hormonal contraception before recruitment into the study. Baseline information about these women is summarized in Table 1. Mean age was just under 18 years and most women were still in full-time education.

The majority of women were African except in the COC group, which included 22% of women of Indian origin and 21% “Colored” (mixed race). More than a quarter of women (29.3%) in the DMPA user group had ever been pregnant compared to low prevalence of pregnancy in the other groups. COC users were more likely to be smokers than other method users, although smoking generally was low across all groups.

In total, 277 women continued with the same method of contraception for at least two follow-up visits, while the rest had changed method or stopped using contraception. Total same-method follow-up time was 648 person-years in these 277 participants. Women who continued in the study using a different method of contraception, or stopped using contraception, were included up to the visit they changed method. Overall, 64% of women continued in the study for between 2 and 3 years and 43% completed between 4 and 5 years of follow-up depending on time of recruitment. Main reasons for discontinuation from the study included pregnancy, illness that could have affected BMD such as tuberculosis and moving out of the area.

Table 2 shows mean BMD and BMI at enrolment for each group. Radius BMD was similar in all four contraceptive user groups at baseline ($p=.196$). The regression model showed that BMI was the only baseline characteristic associated with radius BMD ($p<.001$). BMI increased consistently by $0.11 \text{ kg/m}^2/\text{year}$ (95% CI=0.04 to 0.18) in all user groups with no evidence of differences in growth between groups ($p=.14$).

Table 2

Mean BMD and BMI at admission by contraceptive group

	DMPA (<i>n</i> =115)	NET-EN (<i>n</i> =115)	COC (<i>n</i> =116)	Nonuser (<i>n</i> =144)	p value
Radius BMD (g/cm^2)	0.459 (0.06)	0.446 (0.06)	0.460 (0.05)	0.455 (0.05)	.196
Body weight (kg)	59.1 (12.1)	59.5 (9.8)	56.7 (11.4)	59.7 (11.6)	.13
Height (cm)	154.8 (5.2)	156.1 (5.7)	156.2 (7.5)	155.9 (7.3)	.30
BMI (kg/m^2)	24.63 (4.77)	24.31 (3.75)	23.18 (4.97)	24.61 (5.85)	.097

BMI, body mass index.

Data are expressed as mean $\pm$ SD.

Radius BMD increased in all groups during follow-up (Table 3), even after adjusting for changes in BMI ($p<.001$), with an overall increase for all women combined of 1.39% per annum (95% CI=1.19% to 1.59%). In nonusers, BMD increased by 1.49% per annum; in the DMPA, NET-EN and COC users, the increases were less: 1.39% ($p=.76$), 1.03% ($p=.05$) and 0.84% ($p=.01$), respectively (Table 3). There was moderate to strong evidence for smaller increases per annum in NET-EN ($p=.050$) and COC ($p=.010$) users compared to nonusers. There was no evidence of a difference in BMD between DMPA and nonusers ($p=.76$) (Table 3).

There was no evidence of a difference in change in radius BMD in the two time periods (up to 2.5 years and 2.5 years

Table 1

Sociodemographic, lifestyle and reproductive characteristics of subjects aged 15–19 years by contraceptive user group, at baseline

Characteristics	DMPA (<i>n</i> =115)	NET-EN (<i>n</i> =115)	COC (<i>n</i> =116)	Nonuser controls (<i>n</i> =144)	p value
Mean (SD) age (years)	17.8 (1.4)	17.4 (1.3)	17.8 (1.0)	17.4 (1.2)	.0002
Mean (SD) highest education grade	10.9 (1.5)	10.5 (1.5)	11.3 (0.8)	10.3 (1.4)	.0001
Marital status (%)					.0001
Married/Cohabiting	5.3	5.2	10.3	12.8	
Regular partner—not cohabiting	92.1	83.8	87.1	63.5	
Casual partner	2.6	5.2	2.6	23.0	
No partner	0	0.9	0	0.7	
Employment status (%)					<.0001
Employed full-/part-time	6.2	2.6	9.5	2.7	
Unemployed	11.4	10.5	8.5	7.4	
Student/Scholar	82.5	86.8	79.1	98.8	
Ethnicity (%)					<.0001
African	94	89	57	92	
Colored	5	7	21	4	
Indian	1	4	22	4	
Ever pregnant (%)	29.3	3	6	2	<.0001
Ever lactated (%)	16.7	0.9	2.6	1.4	
Lactation (years) median	<1	<1	<1	<1	
Mean (SD) age at menarche (years)	13.9 (1.3)	13.8 (1.2)	13.6 (1.48)	13.5 (1.29)	.19
Exercise (%)					.0001
No regular exercise	78.1	72.8	64.9	77.7	
At least once a week	21.9	27.2	35.1	22.3	
Dieted in last 6 months (%)	3.5	2.6	5.3	2.0	.54
Ever smoked cigarettes (%)	9.6	11.3	27.3	4.8	<.001
Current smoker (%)	6.0	7.0	13.9	3.4	.006

Table 3

Factors associated with radius BMD at study visit, estimated from random-effects linear regression model

Variable	% change in BMD (95% CI) per annum	% change in BMD per method relative to nonuser (p)	n	Time at risk (years)
Use of contraceptive method, per year ^a				
Nonuser (reference)	1.49 (1.25–1.72)	0	96	311
DMPA	1.39 (0.79–1.98)	–0.10 (.76)	51	76
NET-EN	1.03 (0.63–1.44)	–0.45 (.050)	71	146
COC	0.84 (0.39–1.28)	–0.65 (.010)	59	116
Effect of BMI, per unit change in BMI (% per kg/m ²) ^b	0.267 (0.157–0.377)		277	648

^a Adjusted for BMI.

^b Adjusted for contraceptive method.

and longer) in any of the four study groups (DMPA, $p=.105$; NET-EN, $p=.7$; nonusers, $p=.46$; COC, $p=.06$).

We investigated changes in BMD in a group of 14 NET-EN users who discontinued use of the method without restarting another method during the course of the study. An overall reduction in radius BMD of 0.61% (95% CI=–2.53% to 1.34%) per year was seen in the 14 NET-EN users during use of the method, followed upon cessation of method by an increase of 0.69% (95% CI=–0.18% to 1.56%) per year. After adjusting for BMI, there was some evidence of a difference in rate of BMD change before and after use of NET-EN ($p=.066$). Most COC and DMPA discontinuers restarted other methods; thus, there were too few to conduct a group analysis.

4. Discussion

Our results show that radius BMD increased during follow-up in the nonuser and all three user groups after adjusting for BMI. There was some evidence of less increase in NET-EN and COC groups compared to the nonuser group. Our results confirm those obtained from an interim analysis of this study [15] in regard to NET-EN. However, in the preliminary analysis, COC users did not show any differences in BMD increase from nonusers, possibly because the interim analysis lacked power.

In the only other study that has previously investigated the effect of NET-EN use [14], BMD was measured in the left calcaneus in a large cross-sectional sample of adult premenopausal women. NET-EN users had similar values to DMPA users, and these were both significantly lower compared to never users.

We found no evidence of any change in BMD growth in the period up to 2.5 years of use compared to the period from 2.5 years of use. Several studies have reported that the rate of loss of BMD in DMPA users is greatest in the first 2 years of use [4,12,13,22]. Only one of these studies has investigated rate of change in adolescents [12].

Several longitudinal studies have found that adolescent users of low-dose COCs may gain bone mass at a slower rate compared to nonusers [10,17,18]. The COC formulations in our study were similar to those reported in these studies. Our study also showed that COC users gained BMD at a slower rate than nonusers. Although DMPA users had a lower yearly increase in BMD compared to nonusers, this was not significant. Other studies measuring BMD at central sites such as the hip and spine have found that adolescent DMPA users have lower BMD values compared to nonusers [6–13]. Studies using the forearm as a site of BMD measurement have generally not found significant differences between DMPA users and controls [23,24]. Our study has found similar results to others measuring the forearm with respect to DMPA. The forearm may be more sensitive to the effect of NET-EN and COC and it may be that these results would be magnified if measured at a central site such as the hip or spine. No studies have measured BMD at central sites in NET-EN users.

Our study shows some evidence of a recovery in growth in BMD in a very small sample of NET-EN discontinuers who did not use another method of contraception after cessation. The cross-sectional study that investigated women who commenced NET-EN during adolescence (18 years and younger) and then ceased using the method [14] found no difference in BMD values between those who had ceased use 2–3 years before and never users regardless of whether they were early starters (adolescents) or late starters (post-adolescence). It was not possible to make any statement about BMD recovery in the COC and DMPA discontinuers in our study as numbers were so small.

DMPA suppresses estradiol production, leading to estrogen deficiency [25]. This leads to greater bone resorption and, hence, loss of BMD. There is less information on NET-EN and the mechanisms by which it could affect BMD. Studies on NET alone have not found a negative effect on BMD [26–28]. There are now limited data available on recovery of BMD in women who commence DMPA in adolescence [12,13]. This is the first longitudinal study that has included NET-EN users and discontinuers. Further evidence is needed to ensure that any losses of BMD as a result of DMPA and NET-EN in adolescence are fully recovered and peak bone mass is not compromised. Until that time, the recommendations for DMPA and NET-EN in adolescents will continue to caution long-term use in young women.

The third edition (2004) of the Medical Eligibility Criteria for Contraceptive Use of the WHO [29] has changed the classification of use of DMPA and NET-EN for women below 18 years and above 45 years from Category 1 (no restriction for the use of the contraceptive method) to Category 2 (advantages of using the method generally outweigh the theoretical or proven risks). This is backed up in the document by a number of statements reporting on current evidence of the effect of DMPA on BMD. The WHO has recommended that in the absence of evidence on NET-EN, the same restrictions should apply to NET-EN users.

Our study shows that although BMD continues to increase in adolescent users of NET-EN and COCs, these increases are lower than that in nonusers. Although the percent yearly increase in DMPA users was lower than that in nonusers, there was no evidence of a significant difference and this may have been because this group had the lowest number of years at risk included in the analysis.

4.1. Limitations

Longitudinal studies of adolescent users of hormonal contraception are often limited by method discontinuation. This was the case in our study where few women remained on hormonal contraception for more than 2 years without a change of method or stopping use of contraception altogether. Concerns regarding long-term use of DMPA and NET-EN in adolescents should take this into consideration as many young women will have discontinued use before the 2-year review suggested on the patient labeling. Our study has used the forearm as its site of measurement, which may be less sensitive than the hip and spine to changes in BMD resulting from hormonal contraceptive use.

References

- [1] Petitti DB, Piaggio G, Mehta S, Cravioto MC, Meirik O. Steroid hormone contraception and bone mineral density: a cross-sectional study in an international population. *J Obstet Gynaecol* 2000;95: 736–43.
- [2] Scholes D, Lacroix AZ, Ott SM, Ichikawa LE, Barlow WE. Bone mineral density in women using depot medroxyprogesterone acetate for contraception. *J Obstet Gynaecol* 1999;93:233–8.
- [3] Wanichsetakul P, Kamudhamas A, Watanaruagkovit P, Siripakam Y, Visutakul P. Bone mineral density at various anatomic bone sites in women receiving combined oral contraceptives and depot-medroxyprogesterone acetate for contraception. *Contraception* 2002;65: 407–10.
- [4] Curtis KM, Martins SL. Progestogen-only contraception and bone mineral density. *Contraception* 2006;73:470–87.
- [5] Cundy T, Evans M, Roberts H, Waltie D, Ames R, Reid IR. Bone density in women receiving depot medroxyprogesterone acetate for contraception. *BMJ* 1991;303:13–6.
- [6] Scholes D, Lacroix AZ, Ichikawa LE, Barlow WE, Ott SM. The association between depot medroxyprogesterone acetate contraception and bone mineral density in adolescent women. *Contraception* 2004; 69:99–104.
- [7] Cromer BA, McArdle Blair J, Mahan JD, Zibners BS, Naumovski Z. A prospective comparison of bone density in adolescent girls receiving depot medroxyprogesterone acetate (Depo-Provera), levonorgestrel (Norplant), or oral contraceptives. *J Pediatr* 1996;29:671–6.
- [8] Busen NH, Britt RB, Rianon M. Bone mineral density in a cohort of adolescent women using depot medroxyprogesterone acetate for one to two years. *J Adolesc Health* 2003;32:257–9.
- [9] Lara-Torre E, Edwards CP, Perlman S, Hertweck SP. Bone mineral density in adolescent females using depot medroxyprogesterone acetate. *J Pediatr Adolesc Gynecol* 2004;17:17–21.
- [10] Cromer BA, Stager M, Bonny A, et al. Depot medroxyprogesterone acetate, oral contraceptives and bone mineral density in a cohort of adolescent girls. *J Adolesc Health* 2004;35:434–41.
- [11] Cromer BA, Bonny A, Stager M, et al. Bone mineral density in adolescent females using injectable or oral contraceptives: a 24-month prospective study. *Fertil Steril* 2008 [electronic publication ahead of print].
- [12] Scholes D, Lacroix AZ, Ichikawa LE, Barlow WE, Ott SM. Change in bone mineral density among adolescent women using and discontinuing depot medroxyprogesterone acetate contraception. *Arch Pediatr Adolesc Med* 2005;159:139–44.
- [13] Clark MK, Sowers MR, Levy B. Bone mineral density loss and recovery during 48 months in first-time users of depot medroxyprogesterone acetate. *Fertil Steril* 2006;86:1466–74.
- [14] Rosenberg L, Zhang Y, Constant D, et al. Bone status after cessation of use of injectable progestin contraceptives. *Contraception* 2007;76: 425–31.
- [15] Beksinska ME, Kleinschmidt I, Smit J, Farley TMM. Bone mineral density in adolescents using norethisterone enanthate, depot-medroxyprogesterone acetate, or combined oral contraceptives for contraception. *Contraception* 2007;75:438–43.
- [16] Martins SL, Curtis KM, Glasier AF. Combined hormonal contraception and bone health: a systematic review. *Contraception* 2006;73: 445–69.
- [17] Hartard M, Kleimond C, Wiseman M, Weissenbacher ER, Felsenberg D, Erben RG. Detrimental effect of oral contraceptives on parameters of bone mass and geometry in a cohort of 248 young women. *Bone* 2006;40: 444–50.
- [18] Polatti F, Perotti F, Felippa N, Gallina D, Nappi RE. Bone mass and long-term monophasic oral contraceptive treatment in young women. *Contraception* 1995;51:221–4.
- [19] Kaunitz AM, Miller PD, Montgomery Rice V, Ross D, McClung MR. Bone mineral density in women aged 25–35 years receiving depot-medroxyprogesterone acetate: recovery following discontinuation. *Contraception* 2006;74:90–9.
- [20] Scholes D, LaCroix AZ, Ichikawa LE, Barlow WE, Ott SM. Injectable hormone contraception and bone density: results from a prospective study. *Epidemiology* 2002;13:581–7.
- [21] Nordin BEC. The definition and diagnosis of osteoporosis. *Calcif Tissue Int* 1987;40:57–8.
- [22] Tang OS, Tang G, Yip PS, Li F. Further evaluation on long-term depot-medroxyprogesterone acetate use and bone mineral density; a longitudinal cohort study. *Contraception* 2000;62:161–4.
- [23] Bahamondes L, Perotti M, Castro S, Faundes D, Petta C, Bedone A. Forearm bone density in users of Depo-Provera as a contraceptive method. *Fertil Steril* 1999;71:849–52.
- [24] Tharnprisan W, Taneepanichskul S. Bone mineral density in adolescent and young Thai girls receiving oral contraceptives compared with depot medroxyprogesterone acetate: a cross-sectional study in young Thai women. *Contraception* 2002;66:101–3.
- [25] Ortiz A, Hiroi F, Stanczyk FZ, Goebelsmann V, Mishell DR. Serum medroxyprogesterone acetate (MPA) concentrations and ovarian function following intramuscular injection of Depo-MPA. *J Clin Endocrinol Metab* 1977;44:32–8.
- [26] Eldred JM, Haynes PJ, Thomas EJ. A randomized double blind placebo controlled trial of the effects on bone metabolism of the combination of nafarelin acetate and norethisterone. *Clin Endocrinol* 1992;37:354–9.
- [27] Horowitz M, Wishart JM, Need AG, Morris HA, Nordin BEC. Effects of norethisterone on bone related biochemical variables and forearm bone mineral in postmenopausal osteoporosis. *Clin Endocrinol* 1993; 39:649–55.
- [28] Riis BJ, Christiansen C, Johansen JS, Jacobson J. Is it possible to prevent bone loss in young women treated with luteinizing hormone-releasing hormone agonists? *J Clin Endocrinol Metab* 1990;70: 920–4.
- [29] World Health Organization. Medical eligibility criteria for contraceptive use, third edition. Department of Reproductive Health and Research. Geneva (Switzerland): World Health Organization; 2004.

Original research article

Bone mineral density in young women aged 19–24 after 4–5 years of exclusive and mixed use of hormonal contraception

Mags E. Beksinska^{a,*}, Immo Kleinschmidt^b, Jenni A. Smit^a,
Timothy M.M. Farley^c, Helen V. Rees^a

^aReproductive Health and HIV Research Unit, Department of Obstetrics and Gynaecology, University of the Witwatersrand, Mayville, 4091, South Africa

^bLondon School of Hygiene and Tropical Medicine, Keppel Street, London WC1E

^cUNDP/UNFPA/WHO/World Bank Special Programme of Research, Development and Research Training in Human Reproduction, CH 1211 Geneva 27, Switzerland

Received 15 January 2009; revised 3 February 2009; accepted 3 February 2009

Abstract

Background: Use of depot-medroxyprogesterone acetate (DMPA), norethisterone enanthate (NET-EN) and low-dose combined oral contraceptives (COCs) has been associated with loss of bone mineral density (BMD) in adolescents. However, the effect of using a combination of these methods over time in this age group is limited. The aim of this cross-sectional study was to investigate BMD in young women (aged 19–24 years) with a history of mixed hormonal contraceptive use.

Study Design: BMD was measured at the spine, hip and femoral neck using dual X-ray absorptiometry. Women were classified into three groups: (1) injectable users (DMPA, NET-EN or both) ($n=40$), (2) mixed COC and injectable users ($n=13$) and (3) non-user control ($n=41$).

Results: Women in the injectables-only user group were found to have lower BMDs compared to the non-user group at all three sites, and there was evidence of a difference in BMD between these two groups at the spine after adjusting for body mass index ($p=.042$), hip ($p=.025$) and femoral neck ($p=.023$). The mixed COC/injectable user group BMD values were lower than those for controls; however, there was no evidence of a significant difference between this group and the non-user group at any of the three sites.

Conclusion: This study suggests that BMD is lower in long-term injectable users but not when women have mixed injectable and COC use. © 2009 Elsevier Inc. All rights reserved.

Keywords: Bone mineral density; DMPA; NET-EN; COCs; Adolescents; Young women

1. Introduction

The long-acting progestogen injectable contraceptives depot-medroxyprogesterone acetate (DMPA) and norethisterone enanthate (NET-EN) have been found to have a negative effect on bone mineral density (BMD) in adult premenopausal women [1–5] and in adolescents [6–15]. The effect of combined oral contraceptives (COCs) on BMD has been found to be variable with no effect reported in adult premenopausal women [16], but there is growing evidence that low-dose COCs may be detrimental to BMD in adolescents and young women [10,17,18].

Although recovery of BMD following discontinuation of DMPA is documented in adult premenopausal women [19,20] and in adolescent users [12,13], concerns regarding bone loss in DMPA users resulted in a US Food and Drug Administration Black Box warning for DMPA, stating its use may impair BMD [21]. In the UK, the Medicines and Healthcare Products Regulatory Agency (MHRA) suggests that another contraceptive method be used after 2 years of DMPA use [22]. Much less information is available on recovery in NET-EN users as it is not as widely used as DMPA and not available in the United States or UK. Limited data have found recovery of BMD in adolescent NET-EN users [14,23].

More evidence is needed on long-term use of hormonal contraception and, in particular, on women who switch from hormonal injectables to another hormonal method of contraception. The objective of this study was to determine

* Corresponding author. Tel.: +031 261 8840; fax: +031 261 8868.
E-mail address: mbeksinska@rhu.co.za (M.E. Beksinska).

if long-term use of hormonal contraceptives, including mixed use (COCs and/or, DMPA and/or NET-EN), was associated with a change in bone mass in women aged 19–24 years compared to non-users.

2. Subjects and methods

This cross-sectional study of young women aged 19–24 years measured BMD in the hip, spine and femoral neck. All the women had been recruited 4–5 years previously into a longitudinal study where the BMD in the distal radius had been measured at 6-month intervals. Details of the longitudinal study methodology and results are described elsewhere [15]. At the end of the follow-up period, additional funds allowed for 100 single measurements of the central BMD sites providing an opportunity for measurements at other sites. A subsample of women attending their final longitudinal follow-up visit were informed about this additional component of the study at their last cohort visit and were invited to participate. This subsample included all women completing the study over a 5-month period.

At the start of the 5-year longitudinal study, initiators of DMPA, NET-EN, COCs and non-users of contraception were enrolled from a family planning clinic in Durban, South Africa. All women recruited had no history of hormonal contraceptive use prior to enrolment ($n=490$). The study cohort was recruited between July 2000 and July 2002, and follow-up continued until April 2006. All DMPA users were started on a regimen of 150 mg every 12 weeks and NET-EN users on 200 mg every 8 weeks. Both DMPA and NET-EN were administered intramuscularly. The COCs used by women included a range of formulations, with almost all (93%) using low-dose formulations containing between 30 and 40 mcg of estrogen. Women were eligible to participate if they had not lactated or delivered in the past 6 months, were not currently using and had not used medication known to affect calcium metabolism for more than 3 months, and did not have a chronic disease affecting calcium metabolism. At the baseline and follow-up visits, participants' height, weight and blood pressure were measured using a standard protocol, and a questionnaire was administered to elicit information on demographic characteristics, regularity of the menstrual cycle, smoking, diet, exercise and caffeine and alcohol intake. It was from this cohort that the 100 women were recruited for the cross-sectional study reported here.

History of contraceptive use had been recorded in the longitudinal study over the total follow-up period at approximately six-monthly intervals. Women in the cohort continued the same follow-up schedule even if they stopped, changed or started a contraceptive method. The additional visit to measure the hip, spine and femoral neck BMD was conducted between October 2005 and February 2006. The DEXA equipment used in the cross-sectional substudy to measure hip, spine and femoral neck was a Hologic QDR discovery, version 12.6, model: Discovery w(s/n) 49369

(Hologic Inc, USA). The DEXA equipment was standardized daily using a phantom, and accuracy to the standard during the measurement phase was 0.44%.

The characteristics of women in the study were quantified as means $\pm$ SD, medians or percentages. Differences in BMD and weight between contraceptive groups, and the associations between spine, hip and femoral neck BMD and contraceptive group were assessed using one-way analysis of variance and multiple variable linear regression. Data were analyzed using the statistical package STATA V.10 (College Station, TX, USA).

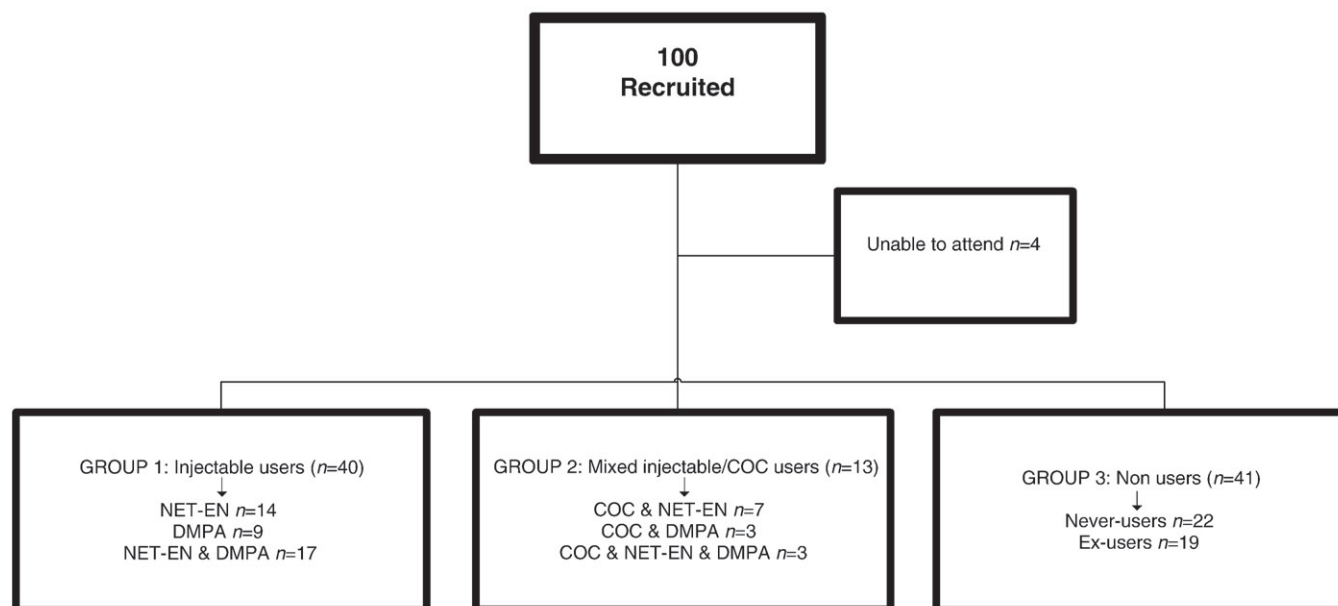
Ethical approval was granted by the University of the Witwatersrand Human Subjects Research Committee (protocol no. M981001) and by the Scientific and Ethical Review Group of the World Health Organization.

3. Results

A group of 100 women who had completed follow-up in the longitudinal phase agreed to participate in the cross-sectional substudy. Of these, 96 women attended their appointment and 4 women were unable to attend due to work commitments. The contraceptive history of these women varied considerably as women had not been discontinued from the longitudinal study if they changed or stopped a method. Thirty women presented with a history of mixed contraceptive use, including women who had used at least two of the study methods (DMPA, NET-EN, COCs) in the preceding 4–5 years. Of these 30, 17 women had used both DMPA and NET-EN, 10 had mixed COCs and either DMPA or NET-EN and 3 women had used all three methods. Far fewer women presented as exclusive users of one method at the end of the follow-up period (DMPA $n=9$, NET-EN $n=14$, COC users $n=2$). The remaining 41 women were non-users of hormonal contraception.

The small number of women who had used one method only made data difficult to analyze, and we therefore classified the women into three broad groups for analysis, according to their contraceptive history over the previous 4–5 years in the longitudinal component of the study. The first group ($n=40$) included all users of injectable hormonal contraception, regardless of whether they had used only one or both of the injection types. The second user group is composed of women who mixed COCs with injections ($n=13$). Finally, the nonhormonal contraceptive users ($n=41$) included 22 never users of hormonal contraception and 19 ex-users. The ex-users had not used a hormonal method for approximately 2 years or longer, and almost all had less than 1 year of lifetime use. These women had started a method on recruitment into the longitudinal study and had subsequently discontinued use. We excluded the two exclusive COC users as they could not be classified into any of the three groups. Fig. 1 shows details of the three groups.

Baseline information about these women is summarized in Table 1. Mean age was approximately 22 years in all



Note: 2 COC exclusive users excluded

Fig. 1. Participant contraceptive history by study analysis group.

groups, and the majority of women were African. Few women participated in any regular exercise, and only one woman was a current smoker. Alcohol consumption was low across all groups.

There was evidence that BMD was associated with body mass index (BMI) for all three sites, with one unit of BMI corresponding to an average increase in BMD of 0.010 (0.006–0.015), 0.014 (0.009–0.017) and 0.017 (0.012–0.021) g/cm² for spine, hip and femoral neck, respectively.

Table 1
Characteristics of subjects aged 19–24 years by contraceptive user group

Characteristics	Injectable users (n=40)	Mixed COC and injectable users (n=13)	Non-users (n=41)	p
Mean age (years) SD	22.3 (1.4)	22.1 (1.2)	22.0 (1.6)	.7
Ethnicity (%)				
African	90.0	100.0	97.6	.5
Colored	7.5	0	2.4	
Indian	2.5	0	0	
Ever pregnant (%)	12.5	0	4.8	.23
Mean age at menarche (years) SD	13.9 (1.5)	13.8 (1.2)	13.7 (1.3)	.47
Exercise at least once a week (%)	5.1	16.7	5.4	.05
Dieted in last 6 months (%)	2.5	0	0	.52
Current smoker (%)	0.0	0	2.6	.51
Alcohol consumption, units per week (%) [*]				
Never	92.5	100.0	73.2	.17
1–2 units	7.5	0	22.0	
3–4 units	0	0	4.9	

^{*} 1 unit=one small glass wine/one measure of spirits.

Table 2 shows mean BMD at the three sites measured. Women in the injectables-only user group were found to have lower BMDs compared to the non-user group at all three sites. There was some evidence of a significant difference in BMD between the injectable and non-user groups at the spine ($p=.042$), hip ($p=.025$) and femoral neck ($p=.023$) after adjusting for BMI. In the mixed COC and injectable group, the BMD values were lower than in the non-users at all sites; however, there was no evidence of a significant difference between this group and the non-users (spine $p=.54$, hip $p=.15$, femoral neck $p=.43$).

Weight in the non-user group remained similar to that at baseline, while women in the injectable user group had gained on average 6.7 kg and the mixed COC/injectable group gained 1.9 kg (data not shown) over the 4- to 5-year follow-up.

4. Discussion

Our results show evidence of significantly lower BMD values in the hip, spine and femoral neck between the adolescent users of injectable hormonal methods and non-users but not those women who have used COCs in combination with injectables. The absence of a significant difference in BMD between this group and the non-users may be due to the small number in this group ($n=13$). Over the follow-up period, this mixed-use group used COCs before and after injectable use and some moved between these methods several times. However, this group had less

Table 2

Mean spine, hip and femoral neck BMD by contraceptive user group relative to non-users

	Injectable users (n=40)	Mixed COC and injectable users (n=13)	Non-users (n=41)
Mean BMD (g/cm ²) (SD)			
Spine	0.944 (0.109) (p=.042)	0.960 (0.094) (p=.54)	0.975 (0.115)
Hip	0.928 (0.116) (p=.025)	0.919 (0.099) (p=.15)	0.954 (0.102)
Femoral neck	0.844 (0.125) (p=.023)	0.855 (0.101) (p=.43)	0.871 (0.130)
Mean BMI (kg/m ²) (SD)	26.4 (4.9) (p=.14)	25.4 (3.1) (p=.71)	24.9 (4.3)

p values from regression model adjusting for BMI.

overall injectable exposure over the follow-up period compared to the injectable-only group.

Although we are unable to comment on any specific method, our sample consisted of a representative group of women completing follow-up in the longitudinal study during a specific time period. At the end of our study, the mixed hormonal contraceptive users were found to be far more common than the exclusive users. Few women stayed on one hormonal method and many had discontinued hormonal contraception altogether. Although the study was not designed to collect reasons for discontinuation of method, anecdotal reports indicated that various side effects and change in relationship status resulted in breaks and changes in method.

The literature has shown that discontinuation rates among adolescent DMPA users are particularly high, and continuation rates as low as 27% at 1 year have been found in the United States [24]. In the longitudinal component of our study, there was an incidence of 37% method change per person-year for DMPA and 26% for NET-EN [15]. Although concerns remain regarding hormonal contraceptive use in adolescents, data indicate that few women are long-term exclusive users of one method of contraception. If mixed users are more prevalent, as was seen in our study, it would be important to include them in studies looking at hormonal contraceptive use and BMD.

Two studies have studied young women who commenced low-dose COCs after use of DMPA and found they were at risk for loss of BMD [25,26]. Our study could not confirm this finding as our numbers using DMPA followed by COCs was too small. In addition, the ethinyl estradiol (EE) formulations used by the COC users in our study was 30–40 mcg, whereas studies that have found a detrimental effect of COCs on BMD have used even lower (20 mcg) EE formulations [10,17,18,25,26]. The MHRA (UK) guidelines [22] recommend changing from DMPA to another method of contraception after 2 years of use; however, there is no advice as to what would be the most suitable method post-discontinuation. As the body of evidence on recovery of BMD in adult and adolescent women post discontinuation of DMPA increases [27], this recommendation may need to be reviewed and balanced against the benefit of using DMPA as a long-term effective contraceptive. The third edition of the WHO Medical Eligibility Criteria for contraceptive Use [28] states the

advantages of using DMPA generally outweigh the theoretical or proven risks in relation to BMD. A position paper published in 2006 by the Society of Adolescent Medicine [29] supports the WHO position on DMPA for woman aged 18–45 years and states that with existing evidence, the advantages of using DMPA in women below 18 years outweigh the disadvantages.

Limited data on recovery of BMD on cessation of hormonal contraceptive use in adolescents have been reported in exclusive users of DMPA [12,13] and NET-EN [14,23]. Further evidence is needed to show that any loss of BMD, resulting from hormonal contraceptive use in adolescence, is fully recovered, and peak bone mass is not compromised. Until this time, the recommendations for DMPA and NET-EN use in adolescents will continue to caution long-term use in young women.

4.1. Limitations

This study was unable to analyze the individual effects of any of the three hormonal methods used as the sample size of exclusive users was too small for analysis.

Acknowledgments

This study was supported by a grant from the World Health Organization, and The William and Flora Hewlett Foundation.

References

- [1] Petitti DB, Piaggio G, Mehta S, Cravioto MC, Meirik O. Steroid hormone contraception and bone mineral density: a cross-sectional study in an international population. *J Obstet Gynaecol* 2000;95: 736–43.
- [2] Scholes D, Lacroix AZ, Ott SM, Ichikawa LE, Barlow WE. Bone mineral density in women using depot medroxyprogesterone acetate for contraception. *J Obstet Gynaecol* 1999;93:233–8.
- [3] Wanichsetakul P, Kamudhamas A, Watanaruagkovit P, Siripakam Y, Visutakul P. Bone mineral density at various anatomic bone sites in women receiving combined oral contraceptives and depot-medroxyprogesterone acetate for contraception. *Contraception* 2002;65: 407–10.
- [4] Curtis KM, Martins SL. Progestogen-only contraception and bone mineral density. *Contraception* 2006;73:470–87.
- [5] Cundy T, Evans M, Roberts H, Waltie D, Ames R, Reid IR. Bone density in women receiving depot medroxyprogesterone acetate for contraception. *BMJ* 1991;303:13–6.

- [6] Scholes D, Lacroix AZ, Ichikawa LE, Barlow WE, Ott SM. The association between depot medroxyprogesterone acetate contraception and bone mineral density in adolescent women. *Contraception* 2004; 69:99–104.
- [7] Cromer BA, McArdle Blair J, Mahan JD, Zibners BS, Naumovski Z. A prospective comparison of bone density in adolescent girls receiving depot medroxyprogesterone acetate (Depo-Provera), levonogestrel (Norplant), or oral contraceptives. *J Pediatr* 1996; 29:671–6.
- [8] Busen NH, Britt RB, Rianon M. Bone mineral density in a cohort of adolescent women using depot medroxyprogesterone acetate for one to two years. *J Adolesc Health* 2003;32:257–9.
- [9] Lara-Torre E, Edwards CP, Perlman S, Hertweck SP. Bone mineral density in adolescent females using depot medroxyprogesterone acetate. *J Pediatr Adolesc Gynecol* 2004;17:17–21.
- [10] Cromer BA, Stager M, Bonny A, et al. Depot medroxyprogesterone acetate, oral contraceptives and bone mineral density in a cohort of adolescent girls. *J Adolesc Health* 2004;35:434–41.
- [11] Cromer BA, Bonny A, Stager M, et al. Bone mineral density in adolescent females using injectable or oral contraceptives: a 24-month prospective study. *Fertil Steril* 2008 Epub ahead of print.
- [12] Scholes D, Lacroix AZ, Ichikawa LE, Barlow WE, Ott SM. Change in bone mineral density among adolescent women using and discontinuing depot medroxyprogesterone acetate contraception. *Arch Pediatr Adolesc Med* 2005;159:139–44.
- [13] Clark MK, Sowers MR, Levy B. Bone mineral density loss and recovery during 48 months in first-time users of depot medroxyprogesterone acetate. *Fertil Steril* 2006;86:1466–74.
- [14] Rosenburg L, Zhang Y, Constant D, et al. Bone status after cessation of use of injectable progestin contraceptives. *Contraception* 2007;76: 425–31.
- [15] Beksinska ME, Kleinschmidt I, Smit J, Farley TMM. Bone mineral density in adolescents using norethisterone enanthate, depot-medroxyprogesterone acetate, or combined oral contraceptives for contraception. *Contraception* 2007;75:438–43.
- [16] Martins SL, Curtis KM, Glasier AF. Combined hormonal contraception and bone health: a systematic review. *Contraception* 2006;73: 445–69.
- [17] Hartard M, Kleimond C, Wiseman M, Weissenbacher ER, Felsenberg D, Erben RG. Detrimental effect of oral contraceptives on parameters of bone mass and geometry in a cohort of 248 young women. *Bone* 2006;40:444–50.
- [18] Polatti F, Perotti F, Felippa N, Gallina D, Nappi RE. Bone mass and long-term monophasic oral contraceptive treatment in young women. *Contraception* 1995;51:221–4.
- [19] Kaunitz AM, Miller PD, Montgomery Rice V, Ross D, McClung MR. Bone mineral density in women aged 25–35 years receiving depot-medroxyprogesterone acetate: recovery following discontinuation. *Contraception* 2006;74:90–9.
- [20] Scholes D, LaCroix AZ, Ichikawa LE, Barlow WE, Ott SM. Injectable hormone contraception and bone density: results from a prospective study. *Epidemiology* 2002;13:581–7.
- [21] Black Box warning added concerning long-term use of Depo-Provera contraceptive injection. FDA Talk Paper T04-50, November 17, 2004. <http://www.fda.gov/bbs/topics/ANSWERS/2004/ANS01325.html> (accessed October 2, 2006).
- [22] Faculty of Family Planning and Reproductive Health Care of The Royal College of Obstetricians and Gynaecologists. 2004, Statement on MHRA guidance on Depo-Provera for prevention of postmenopausal bone loss. FFPRHC 2004. http://www.ffprhc.org.uk/admin/uploads/Depo_Provera_alert_statement.pdf (accessed October 2, 2006).
- [23] Beksinska ME, Kleinschmidt I, Smit J, Farley TMM. Bone mineral density in a cohort of adolescents during use of norethisterone enanthate, depot-medroxyprogesterone acetate, or combined oral contraceptives and after discontinuation of norethisterone enanthate. *Contraception* 2009;79:345–9.
- [24] Polaneczky M, Liblanc M. Long-term depot medroxyprogesterone acetate (Depo-Provera) use in inner-city adolescents. *J Adolesc Health* 1998;2:81–8.
- [25] Albertazzi P, Bottazzi M, Steel S. Bone mineral density and depot medroxyprogesterone acetate. *Contraception* 2006;73:577–83.
- [26] Berenson AB, Rahman M, Breitkopf CR, Bi LX. Effects of depot medroxyprogesterone acetate and 20-microgram oral contraceptives on bone mineral density. *Obstet Gynecol* 2008;112:788–99.
- [27] Kaunitz AM, Arias R, McClung M. Bone density recovery after depot medroxyprogesterone acetate injectable contraception use. *Contraception* 2008;77:67–76.
- [28] World Health Organisation. Medical eligibility criteria for contraceptive use Third edition. Geneva 27 Switzerland: Department of Reproductive Health and Research, World Health organization, CH-1211; 2004.
- [29] Society for Adolescent Medicine. Depot medroxyprogesterone acetate and bone mineral density in adolescents — the Black Box warning: a position paper of the Society for Adolescent Medicine. *J Adolesc Health* 2006;39:296–301.

Original research article

Bone mineral density in adolescents using norethisterone enanthate, depot-medroxyprogesterone acetate or combined oral contraceptives for contraception[☆]

Mags E. Beksinska^{a,*}, Immo Kleinschmidt^{b,1}, Jenni A. Smit^a, Timothy M.M. Farley^c^aReproductive Health and HIV Research Unit, Department of Obstetrics and Gynaecology, University of the Witwatersrand, Mayville 4091, South Africa^bMedical Research Council, Durban 4091, South Africa^cDepartment of Reproductive Health and Research, UNDP/UNFPA/WHO/World Bank Special Programme of Research, Development and Research Training in Human Reproduction, World Health Organization, CH 1211 Geneva 27, Switzerland

Received 3 October 2006; revised 10 January 2007; accepted 15 February 2007

Abstract

Purpose: Most studies have shown a negative effect of depot-medroxyprogesterone acetate (DMPA) on the bone mineral density (BMD) of adolescents. There is no information available on the effect of norethisterone enanthate (NET-EN) on BMD in adolescents and the effect of combined oral contraceptives (COCs) on adolescent BMD is inconclusive. The aim of this longitudinal study was to investigate BMD in adolescent (aged 15–19 years) new users of hormonal contraception (DMPA, NET-EN and COCs).

Method: New users of DMPA ($n = 115$), NET-EN ($n = 115$), COCs ($n = 116$) and 144 nonuser controls were recruited. BMD was measured at the distal radius and midshaft of the ulna using dual X-ray absorptiometry.

Results: In total, 275 women were included in this interim analysis and total follow-up time was 553 person-years. There was no significant difference in radius BMD between users of different contraceptive methods at baseline ($p = .40$). Overall, an increase in radius BMD of 0.00522 per person-year was observed. This result was similar when adjusting for BMI in the random effects regression model ($p = .88$). The regression model showed that BMI was significantly associated with radius BMD, with each unit increase in BMI corresponding to an increase of 0.0029 g/cm² in BMD (95% CI 0.0023 to 0.0036, $p < .001$). Interaction between contraceptive method and follow-up time adjusted for BMI was not significant ($p = .07$). The increase in BMD for NET-EN users of 0.0013 g/cm² per person-year (95% CI –0.0017 to 0.0043) was significantly lower than that of nonusers ($p = .017$). For DMPA and COC users, the increase in BMD was not significantly different compared to the nonusers. This study suggests that NET-EN users had lower increase in BMD over time compared to the other user groups.

© 2007 Elsevier Inc. All rights reserved.

Keywords: Bone mineral density; Depot-medroxyprogesterone acetate; Norethisterone enanthate; Combined oral contraceptives; Adolescents

1. Introduction

Studies undertaken to investigate the effect of hormonal contraception on bone mineral density (BMD) have mostly found that depot-medroxyprogesterone acetate (DMPA) has

a negative effect on bone mass [1–5], although some have shown minimal or no effect [6–8]. There is limited information available on the effect of norethisterone enanthate (NET-EN) on BMD [9]. The evidence for the effect of combined oral contraceptives (COCs) in premenopausal women has shown that in general COCs are not associated with changes in BMD [10–12].

A number of studies have investigated the effect of hormonal contraceptives on BMD in adolescents aged less than 20 years [13–19] and almost all of these have found that DMPA has an adverse effect on BMD [14–19]. No studies have looked at adolescent NET-EN users. Emerging data in young COC users are showing that BMD may be

[☆] This study was supported by a grant from the World Health Organization, The Flora and William Hewlett Foundation and The Department for International Development UK (DFID).

* Corresponding author. Tel.: +27 031 261 8840; fax: +27 031 261 8868.

E-mail address: m.beksinska@thru.co.za (M.E. Beksinska).

¹ Current affiliation: London School of Hygiene and Tropical Medicine, Department of Epidemiology and Population Health, Keppel Street, London WC1E 7HT, UK.

compromised in users of low-dose COCs [12,19,20]. Studies of adolescent users of hormonal contraception have often been limited by small sample sizes [15,16] and follow-up in longitudinal studies on adolescents is often poor due to method discontinuation [16,17].

DMPA and NET-EN are effective contraceptives with low failure rates [21]. In South Africa, use of both hormonal injectable contraceptives is high amongst adolescents, with about 70% of Black African women aged 15–19 years practicing contraception using either DMPA or NET-EN [22]. Adolescence is a period of rapid growth and women gain 40–50% of their skeletal mass during this time [23]. Loss of bone mass as a result of hormonal contraceptive use may have more serious implications in these women who have yet to achieve peak bone mass. It would be important to know whether using hormonal methods of contraception interferes with the process of bone mineralization. The objective of the study was to determine whether long-term use of hormonal contraceptives (COCs, DMPA and NET-EN) compared to nonuse is associated with a change in bone mass in women aged 15–19 years.

2. Subjects and methods

A cohort of adolescent never-users of hormonal contraception aged 15 to 19 years starting use of DMPA, NET-EN, COCs, and nonusers of hormonal contraception were recruited from a large family planning clinic in Durban, South Africa. All eligible women were informed about the study and invited to participate if they wished. Recruitment commenced in July 2000 and was completed in 2003. For inclusion into the study, women had to have no history of hormonal contraceptive use. All DMPA users were started on a regimen of 150 mg every 12 weeks and NET-EN users on 200 mg every 8 weeks. Both DMPA and NET-EN were administered intramuscularly. The COCs used by women included a range of formulations, with most (93%) using formulations containing between 30 and 40 µg of estrogen. Women were eligible to participate if they had not lactated or delivered in the past 6 months; were not currently or had never used medication known to affect calcium metabolism for more than 3 months; and did not have a chronic disease affecting calcium metabolism. On recruitment, a questionnaire was administered by a trained research nurse to elicit information on lifetime contraceptive history, fertility history and regularity of the menstrual cycle. Questions were also asked on smoking, diet, exercise and caffeine and alcohol intake. The examination included height, weight and blood pressure using a standard protocol.

Body mass index (BMI) was calculated from the weight and height as kilograms per square meter. Forearm BMD was measured by dual energy X-ray absorptiometry (DEXA model DTX-200, Osteometer MediTech A/S Co, Rodovre, Denmark). BMD was measured in grams per square centimetre at two distal sites in the forearm (radius and ulna). The

distal site contains approximately 25% trabecular bone and 75% cortical bone. The DEXA equipment was standardized daily using a phantom as per manufacturers instructions. Accuracy to the standard during the recruitment and follow-up period was 0.53%, and in vivo precision was 0.94%.

Study participants are being followed-up at six monthly intervals for a total of 5 years. Forearm BMD, height and weight were measured at each follow-up visit. The findings presented in this paper are based on an interim analysis and data collected up to the end of 2004 are included. Those recruited early in the study had completed over 3 years of follow up; however, those recruited in 2002 have approximately 18 months of follow-up. Women were not excluded from the study if they stopped, changed or started a contraceptive method. This was done to investigate issues of recovery of BMD post discontinuation of a hormonal method. The method of contraception used was recorded at each follow-up visit. Women were assigned to the contraceptive method they were using until their next follow-up visit. If at any visit a change was recorded, the woman would be assigned according to the new contraceptive method. Thus, each woman could contribute to more than one of the exposure categories.

This interim analysis is conducted on all women who remained on the same method for at least two visits inclusive of the baseline visit (minimum 6 months) and data are included up to the time the method was changed.

Ethical approval was granted by the University of the Witwatersrand, Human Subjects Research Committee, and by the Scientific and Ethical Review Group of the World Health Organization.

2.1. Statistical analysis

The characteristics of women in the study were quantified as means±SD, medians or percentages. The study was powered to be able to detect a half standard deviation difference in bone mass between users and nonusers of hormonal contraceptives. This could be of biological significance as this difference would translate into a large difference in the risk of fracture in the older woman. Information on the mean and standard deviation of cross-sectional measurements of forearm bone mass in white, European, premenopausal women reported by Nordin [24] was used to estimate sample size. A sample of 110 participants per contraceptive group was required assuming a two-tailed statistical test with a significance level of 5%, power of 80%, and allowing for 10–15% loss to follow-up per year. The nonuser group was over-recruited compared to other groups as in addition to loss to follow-up it was anticipated that some women would commence a method of contraception or become pregnant.

Differences in BMD between contraceptive groups, and the associations between BMD and selected characteristics of the study participants by contraceptive group were assessed using one-way analysis of variance and multiple variable linear regression.

Table 1

Sociodemographic, lifestyle and reproductive characteristics of subjects aged 15–19 years by contraceptive user group, at baseline

Characteristics	DMPA (n=115)	NET-EN (n=115)	COC (n=116)	Nonuser controls (n=144)	p value
Mean age, years (SD)	17.8 (1.4)	17.4 (1.3)	17.8 (1.0)	17.4 (1.2)	.0002
Mean highest education grade (SD)	10.9 (1.5)	10.5 (1.5)	11.3 (0.8)	10.3 (1.4)	.0001
Marital status, %					
Married/cohabiting	5.3	5.2	10.3	12.8	.0001
Reg partner-not cohabiting	92.1	83.8	87.1	63.5	
Casual partner	2.6	5.2	2.6	23.0	
No partner	0	0.9	0	0.7	
Employment status, %					
Employed full/part-time	6.2	2.6	9.5	2.7	<.0001
Unemployed	11.4	10.5	8.5	7.4	
Student/scholar	82.5	86.8	79.1	98.8	
Ethnicity, %					<.0001
African	94	89	57	92	
Colored	5	7	21	4	
Indian	1	4	22	4	
Ever pregnant, %	29.3	3	6	2	<.0001
Ever lactated, %	16.7	0.9	2.6	1.4	
Lactation (yrs), median	<1	<1	<1	<1	
Mean age at menarche, years (SD)	13.9±1.3	13.8±1.2	13.6±1.48	13.5±1.29	.19
Exercise					
No regular exercise, %	78.1	72.8	64.9	77.7	.0001
At least once a week, %	21.9	27.2	35.1	22.3	
Dieted in last 6 months, %	3.5	2.6	5.3	2.0	.54
Ever smoked cigarettes, %	9.6	11.3	27.3	4.8	>.00001
Current smoker, %	6	7.0	13.9	3.4	.006

DMPA=depot medroxyprogesterone acetate; NET-EN= norethisterone enanthate; COC=combined oral contraceptive, SD=standard deviation.

Follow-up of all women was cumulated up to their last visit, or up to the visit preceding a visit at which they reported a change in contraceptive method, whichever occurred first. To assess the effect of contraceptive method on radius BMD, and to control for the confounding effect of changes in BMI, a random effects linear regression model was set up using STATA (V.8, College Station, TX, USA) with radius BMD at the time of a study visit as the response variable, and contraceptive method, follow-up since last study visit and BMI at time of the visit as explanatory variables, and allowing for within-subject correlation of responses. Differences in rate of change in radius BMD per year between method were assessed by including interaction terms in the model for method and follow-up time.

3. Results

In total, 490 women aged 15–19 years were recruited. All women including those in the DMPA, NET-EN and COC groups had no past use of hormonal contraception before recruitment into the study. Baseline information about these women is summarized in Table 1. Mean age was 18, with the DMPA and COC groups being 4 months older than the

nonuser and NET-EN groups ($p=.0002$). Almost all women were African except in the COC group which included about a quarter (22%) of Indian women. This resulted in a significant difference in ethnicity between the groups. Incidence of smoking was low across all groups.

The reproductive characteristics of the woman are summarized in Table 1. Few women had ever been pregnant (teenage pregnancies) except in the DMPA group with a quarter of women (29%) reporting that they were ever pregnant. Age of menarche was similar in all groups and at recruitment; all had regular menstrual cycles, between 21 and 35 days' duration (data not shown).

Table 2 shows mean BMD at both sites and BMI at enrolment for each group. There was no significant difference in radius ($p=.196$) and ulna ($p=.0534$) BMD between the four contraceptive user groups at baseline. The COC group had a slightly lower mean BMI at baseline but this was not significant.

Table 3 shows the baseline mean BMD of women by age at enrolment. The increase in radius and ulna BMD of approximately 3% per year of age was significant.

In this interim analysis period, 401 women had attended at least once for follow-up (82%). Of these women,

Table 2

Mean BMD and BMI at admission by contraceptive group

	DMPA n=115	NET-EN n=115	COC n=116	Nonuser n=144	p value
Radius BMD g/cm ² (SD)	0.459 (0.06)	0.446 (0.06)	0.460 (0.05)	0.455 (0.05)	.196
Ulna BMD g/cm ² (SD)	0.443 (0.06)	0.432 (0.06)	0.429 (0.05)	0.436 (0.06)	.0534
Mean BMI (kg/m ²) (SD)	24.63 (4.77)	24.31 (3.75)	23.18 (4.9)	24.61 (5.8)	.097

Table 3

Mean BMD and BMI by year of age in 15–19 age group at admission

	15, n=36	16, n=57	17, n=101	18, n=173	19, n=123	p value
Radius g/cm ² (SD)	0.412 (0.049)	0.443 (0.054)	0.457 (0.053)	0.455 (0.52)	0.472 (0.056)	<.00001
Ulna g/cm ² (SD)	0.405 (0.054)	0.419 (0.053)	0.440 (0.053)	0.436 (0.053)	0.452 (0.062)	<.00001
Mean BMI (kg/m ²) (SD)	23.59	22.97	24.23	24.26	24.89	.169

31 presented with criteria that made them ineligible to continue in the study. Pregnancy was the main reason for discontinuation and this was high in the nonuser control group. In total, 76% ($n=370$) of women completed their second visit. Main reasons for discontinuation from the study included pregnancy, illness that could have affected BMD, e.g., tuberculosis, and moving out of the area. Of those who continued participation in the study, 50% changed, stopped or started a method at least once during their follow-up. Some women (15%) changed, stopped or started a new method at least twice. Incidence of method change per year is shown in Table 4. Reasons given for method change were mainly change in relationship status or complaints about side effects of the method. This was lowest in the nonuser group where 19% started a method of contraception during their follow-up and highest in the DMPA group where 74% changed or stopped use. DMPA users often changed to NET-EN which is the more popular choice amongst young women.

In total, 275 women had continued the same method for at least 6 months (two visits). Some women had contributed seven study visits (approximately 3.5 years). Total follow-up time was 553 person-years for 275 participants (Table 4).

Overall, an increase in radius BMD of 0.00522 per person-year was observed. Mean BMD of the women in this analysis was 0.45 g/cm² (SD=0.053) at baseline. There was no significant difference in radius BMD between prospective users of different contraceptive methods at baseline ($p=.40$) (Table 4). This result was similar when adjusting for BMI in the random effects regression model ($p=.88$). The regression model showed that BMI was significantly associated with radius BMD, with each unit increase in BMI corresponding to an increase of 0.0029 g/cm² in BMD (95% CI 0.0023 to 0.0036, $p<.001$) (Table 5). Interaction between contraceptive method and follow-up time adjusted for BMI was marginally nonsignificant overall ($p=.07$). For DMPA users, the increase in radius BMD of 0.0069 g/cm² per person-year (95% CI 0.0028 to 0.011) was nonsignificantly different compared to

the increase of 0.0055 g/cm² per person-year (95% CI 0.0037 to 0.0073) for nonusers ($p=.55$). Similarly, the increase of 0.0053 g/cm² per person-year (95% CI 0.0022 to 0.0085) for COC users was nonsignificantly different from the controls. The increase in BMD for NET-EN users of 0.0013 g/cm² per person-year (95% CI -0.0017 to 0.0043) was significantly lower than that of nonusers ($p=.017$), and not inconsistent with the null hypothesis of no increase in BMD over time for these women ($p=.40$).

4. Discussion

Women in the 15–19-year age group have yet to reach peak bone mass and our baseline data indicate that there is a small yearly increase of approximately 2–3% per year in a population that had no exposure to hormonal methods of contraception on recruitment into the study. This increase would be expected in women in this age range [23] and our data indicate that forearm BMD is sensitive enough to document this increase.

The multiple variable analysis shows that BMD is strongly related to BMI ($p<.001$). BMD in the nonuser reference group, the DMPA user group and the COC user group increased significantly during follow-up, by similar amounts (0.0055, 0.0069 and 0.0053 g/cm² per year, respectively) after adjusting for BMI. By contrast, the NET-EN group differed significantly from the nonuser group ($p=.017$), achieving no significant growth in BMD during follow-up. This is the first study to date that has investigated the effect of NET-EN on BMD. NET-EN was the only group where there was no significant increase in BMD found over time ($p=.4$). No significant difference was found between either the COC and the nonuser control group or the DMPA and the nonuser control group.

Our study has used the forearm as the site of measurement and it may be that differences in the lumbar spine and hip are more sensitive and any decreases are magnified at these sites. Other studies [7,13] using the forearm as the site of measurement found no significant differences between

Table 4

Radius BMD at baseline, follow-up time and incidence of method change by contraceptive group

Method	N	Follow-up time in person years	Incidence of method change per person-year	Radius BMD g/cm ² (unadjusted) at baseline	p value
				(SD)	
DMPA	51	75.42	0.37	0.46 (0.06)	.40
NET-EN	68	125.68	0.26	0.44 (0.06)	
Nonuser	96	248.65	0.004	0.45 (0.05)	
COC	60	103.67	0.19	0.46 (0.05)	
Total	275	553			

Table 5

Factors associated with radius BMD at study visit, estimated from random effects liner regression model

Variable	Effect on radius BMD	95% Confidence interval	p
BMI, change in radius BMD per unit change in BMI (g/cm ² per kg/m ²)	0.0029	0.0023 to 0.0036	<.001
Method relative to nonuser (g/cm ²)			
Nonuser (reference)	0		
DMPA,	0.0058	-0.011 to 0.022	.69
NET-EN	-0.0022	-0.017 to 0.013	.77
COC	0.0026	-0.013 to 0.018	.74
Change in radius BMD over time (g/cm ² per person-year) ^a			
Nonuser	0.0055	0.0037 to 0.0073	<.001
DMPA,	0.0069	0.0028 to 0.011	.001
NET-EN	0.0013	-0.0017 to 0.0043	.40
COC	0.0053	0.0022 to 0.0085	.001

In a model without BMI, interaction between method and follow-up time was significant with $p=.036$.

^a Interaction effect of change in radius BMD over time and contraceptive user groups was marginally significant, $p=.07$.

DMPA use and controls. Studies finding decreases in BMD in users of DMPA have mainly used the hip and spine for measurement of BMD.

The loss of BMD in the case of DMPA use has been associated with estrogen deficiency [25]. Some studies show that these negative changes may be reversible and there is a return to pre-use levels on discontinuation of DMPA [1,26]. In addition, there seems to be recovery of BMD in long-term users of DMPA [1,6]. However, there are no long-term data on women who commence DMPA in adolescence and, therefore, there is a need to investigate whether the recovery seen in older age groups applies to this younger group whose BMD may be compromised prior to achieving peak bone mass. It has been suggested that norethisterone (NET) on its own may protect against calcium loss via a direct topic effect on bone or by the action of estrogenic metabolites.

The studies that have included oral NET [27–29] have shown a positive effect on BMD; however, NET-EN may be different in that the depot characteristics are added to this compound through the addition of EN. The lack of data on the effect of NET-EN on BMD may be due to the method not being as widely used internationally as DMPA. However, in South Africa, this method has increased in popularity in recent years and is often the method of choice for younger women [22,30].

In 2004, the US FDA required the addition of a black box warning in the product labeling stating that DMPA use may impair BMD [31] and adolescents were mentioned as a group that could be particularly at risk of bone loss. The following year the WHO issued a statement on hormonal contraception and bone health [32]. Recommendations with regard to bone metabolism are that there should be no restriction on the use of DMPA and its duration of use

in women aged 18–45 years. Evidence around potential safety concerns for women under 18 years using DMPA was not considered adequate at the time and therefore the WHO stated that the advantages of using DMPA outweighed the concerns regarding fracture risk. Recommendations for DMPA applied to NET-EN use. No restriction was placed on use of other progestogen-only or combined hormonal contraceptive methods. A position paper published in 2006 by the Society of Adolescent Medicine [33] supports the WHO position on DMPA use for women aged 18–45 years and states that with existing evidence, the advantages of using DMPA in women below 18 years outweigh the disadvantages. The lack of evidence on the consequences of long-term use of DMPA was acknowledged, and until data became available, it was recommended that prescribing DMPA to adolescents should be considered on a case-by-case basis. A recently published paper [34] has cautioned against the recommendation made by the UK Medicines and Healthcare Products Regulatory Agency (MHRA) [35] that DMPA users should switch to another method of contraception after 2 years of use. Data in the Albertazzi et al. [34] paper shows that women using COCs after DMPA may be particularly at risk for loss of BMD. This present study suggests that DMPA and COC do not affect BMD in younger women, but raises questions about the effect of NET-EN on BMD in younger users.

Future analysis on completion of our study will include information from a larger sample with longer follow-up and may clarify the effect of NET-EN further. In addition, we will be able to examine potential recovery in those who stop using hormonal contraceptive methods. A subsample of women in our study will have hip and spine BMD measurements, but these data are not yet available.

The main limitations to our study were that long-term follow-up in our adolescent women has been affected by frequent method change and discontinuation. Our study has used the forearm as the site of measurement which may be less sensitive than the hip and spine to changes in BMD resulting from hormonal contraceptive use.

References

- [1] Petitti DB, Piaggio G, Mehta S, Cravioto MC, Meirik O. Steroid hormone contraception and bone mineral density: a cross-sectional study in an international population. *J Obstet Gynaecol* 2000;95: 736–43.
- [2] Scholes D, Lacroix AZ, Ott SM, Ichikawa LE, Barlow WE. Bone mineral density in women using depot medroxyprogesterone acetate for contraception. *J Obstet Gynaecol* 1999;93:233–8.
- [3] Wanichsetakul P, Kamudhamas A, Watanaruagkovit P, Siripakam Y, Visutakul P. Bone mineral density at various anatomic bone sites in women receiving combined oral contraceptives and depot-medroxyprogesterone acetate for contraception. *Contraception* 2002; 65:407–10.
- [4] Curtis KM, Martins SL. Progestogen-only contraception and bone mineral density. *Contraception* 2006;73:470–87.
- [5] Cundy T, Evans M, Roberts H, Waltie D, Ames R, Reid IR. Bone density in women receiving depot medroxyprogesterone acetate for contraception. *BMJ* 1991;303:13–6.

- [6] Taneepanichskul S, Intaraprasert S, Theppisai U, Chaturachirda K. Bone mineral density in long-term depot-medroxyprogesterone acetate acceptors. *Contraception* 1997;56:153–5.
- [7] Bahamondes L, Perotti M, Castro S, Faundes D, Petta C, Bedone A. Forearm bone density in users of Depo-Provera as a contraceptive method. *Fertil Steril* 1999;71:849–52.
- [8] Gbolade B, Ellis S, Murphy B, Randall S, Kirkman R. Bone density amongst long term users of medroxyprogesterone acetate. *Br J Obstet Gynaecol* 1998;105:790–4.
- [9] Beksinska M, Smit J, Kleinschmidt I, Farley T, Mbatha F. Bone mineral density in women aged 40–49 years using depot-medroxyprogesterone acetate, norethisterone enanthate or combined oral contraceptives for contraception. *Contraception* 2005;71:170–5.
- [10] Nappi C, Di S, Spiezio SA, Acunzo G, et al. Effects of low-dose and ultra low-dose combined oral contraceptive use on bone turnover and bone mineral density in young fertile women: a prospective controlled randomized study. *Contraception* 2003;67:355–9.
- [11] Paoletti AM, Orru M, Lello S, et al. Short-term variations in bone remodelling markers of an oral contraception formulation containing 3 mg of drospirenone plus 30 microg of ethinyl estradiol: observational study in young postadolescent women. *Contraception* 2004;70:293–8.
- [12] Martins SL, Curtis KM, Glasier AF. Combined hormonal contraception and bone health: a systematic review. *Contraception* 2006;73:445–69.
- [13] Tharnprisarn W, Taneepanichskul S. Bone mineral density in adolescent and young Thai girls receiving oral contraceptives compared with depot medroxyprogesterone acetate: a cross-sectional study in young Thai women. *Contraception* 2002;66:101–3.
- [14] Scholes D, Lacroix AZ, Ichikawa LE, Barlow WE, Ott SM. The association between depot medroxyprogesterone acetate contraception and bone mineral density in adolescent women. *Contraception* 2004;69:99–104.
- [15] Cromer BA, McArdle Blair J, Mahan JD, Zibners BS, Naumovski Z. A prospective comparison of bone density in adolescent girls receiving depot medroxyprogesterone acetate (Depo-Provera), levonogestrel (Norplant), or oral contraceptives. *J Pediatr* 1996;29:671–6.
- [16] Busen NH, Britt RB, Rianon M. Bone mineral density in a cohort of adolescent women using depot medroxyprogesterone acetate for one to two years. *J Adolesc Health* 2003;32:257–9.
- [17] Lara-Torre E, Edwards CP, Perlman S, Hertweck SP. Bone mineral density in adolescent females using depot medroxyprogesterone acetate. *J Pediatr Adolesc Gynecol* 2004;17:17–21.
- [18] Scholes D, Lacroix AZ, Ichikawa LE, Barlow WE, Ott SM. Change in bone mineral density among adolescent women using and discontinuing depot medroxyprogesterone acetate contraception. *Arch Pediatr Adolesc Med* 2005;159:139–44.
- [19] Cromer BA, Stager M, Bonny A, et al. Depot medroxyprogesterone acetate, oral contraceptives and bone mineral density in a cohort of adolescent girls. *J Adolesc Health* 2004;35:434–41.
- [20] Hartard M, Kleimond C, Wiseman M, Weissenbacher ER, Felsenberg D, Erben RG. Detrimental effect of oral contraceptives on parameters of bone mass and geometry in a cohort of 248 young women. *Bone* 2007;40:444–50.
- [21] World Health Organization. World Health Organization Special Programme of Research, Development and Research Training in Human Reproduction. Task Force on Long-Acting Systemic Agents for the Regulation of Fertility. Multinational comparative clinical evaluation of two long-acting injectable contraceptive steroids: norethisterone enanthate given in two dosage regimens and depot medroxyprogesterone acetate: final report. *Contraception* 1983;23:1–20.
- [22] Department of Health South Africa, Medical Research Council and Measure DHS+. South African Demographic and Health Survey 2003, Preliminary Report. Pretoria (South Africa): Department of Health; 2006.
- [23] Sabatier JP, Guaydier-Souquieres G, Laroche D, et al. Bone mineral acquisition during adolescence and early childhood: a study of 574 healthy females 10–24 years of age. *Osteoporos Int* 1966;6:141–8.
- [24] Nordin BEC. The definition and diagnosis of osteoporosis. *Calcif Tissue Int* 1987;40:57–8.
- [25] Ortiz A, Hiroi F, Stanczyk FZ, Goebelsmann V, Mishell DR. Serum medroxyprogesterone acetate (MPA) concentrations and ovarian function following intramuscular injection of depo-MPA. *J Clin Endocrinol Metab* 1977;44:32–8.
- [26] Kaunitz AM, Miller PD, Montgomery Rice V, Ross D, McClung MR. Bone mineral density in women aged 25–35 years receiving depot-medroxyprogesterone acetate: recovery following discontinuation. *Contraception* 2006;74:90–9.
- [27] Eldred JM, Haynes PJ, Thomas EJ. A randomized double blind placebo controlled trial of the effects on bone metabolism of the combination of nafarelin acetate and norethisterone. *Clin Endocrinol* 1992;37:354–9.
- [28] Horowitz M, Wishart JM, Need AG, Morris HA, Nordin BEC. Effects of norethisterone on bone related biochemical variables and forearm bone mineral in postmenopausal osteoporosis. *Clin Endocrinol* 1993;39:649–55.
- [29] Riis BJ, Christiansen C, Johansen JS, Jacobson J. Is it possible to prevent bone loss in young women treated with lutenizing hormone-releasing hormone agonists? *J Clin Endocrinol Metab* 1990;70:920–4.
- [30] Smit J, Gray AL, McFadyen ML, Zuma K. Counting the costs: comparing depot medroxyprogesterone acetate and norethisterone enanthate utilisation patterns in South Africa. *BMC Health Serv Res* 2001;1:4.
- [31] U.S. Food and Drug Administration. Black Box warning added concerning long-term use of Depo-Provera contraceptive injection. FDA Talk Paper T04-50 November 17, 2004. <http://www.fda.gov/bbs/topics/ANSWERS/2004/ANS01325.html> [accessed October 2, 2006].
- [32] Department of Reproductive Health and research. WHO statement on hormonal contraception and bone health. *Contraception* 2006;73:443–4.
- [33] Society for Adolescent Medicine. Depot medroxyprogesterone acetate and bone mineral density in adolescents — The Black Box warning: a position paper of the Society for Adolescent Medicine. *J Adolesc Health* 2006;39:296–301.
- [34] Albertazzi P, Bottazzi M, Steel SA. Bone mineral density and depot medroxyprogesterone acetate. *Contraception* 2006;73:577–83.
- [35] Faculty of Family Planning and Reproductive Health Care of The Royal College of Obstetricians and Gynaecologists. Statement on MHRA guidance on Depo-Provera. FF PRHC 2004. http://www.ffprhc.org.uk/admin/uploads/Depo_Provera_alert_statement.pdf [accessed October 2, 2006].

Original research article

Bone mineral density in women aged 40–49 years using depot-medroxyprogesterone acetate, norethisterone enanthate or combined oral contraceptives for contraception

Mags E. Beksinska^{a,*}, Jenni A. Smit^a, Immo Kleinschmidt^b,
Timothy M.M. Farley^c, Fikile Mbatha^a

^aReproductive Health Research Unit, Department of Obstetrics and Gynaecology, University of the Witwatersrand, Durban, 4001 South Africa

^bMedical Research Council, Malaria Research Programme, 491 Ridge Road, Durban, 4091 South Africa

^cSpecial Programme for Research, Development and Research Training in Human Reproduction, Department of Reproductive Health and Research, World Health Organization, CH-1211 Geneva 27, Switzerland

Received 28 July 2004; revised 25 August 2004; accepted 3 September 2004

Abstract

Most studies show that depot-medroxyprogesterone acetate (DMPA) has a negative effect on bone mass. There are conflicting reports with respect to recovery of bone mass with long-term use of DMPA. No information is available on the effect of norethisterone enanthate (NET-EN) on bone mass, and combined oral contraceptives (COCs) have not been found to be associated with loss of bone mass. The aim of this study was to investigate bone mineral density (BMD) in older women (40–49 years) in relation to use of DMPA, NET-EN and COCs for at least 12 months preceding recruitment into the study.

One-hundred twenty-seven users of DMPA, 102 NET-EN users and 106 COC users were compared to 161 nonuser controls. Bone mineral density was measured at the distal radius and midshaft of the ulna using dual X-ray absorptiometry.

There was no significant difference in BMD between the four contraceptive user groups ($p=.26$) with and without adjustment for age. Although a small decrease in BMD was noted in the age range of 40–49 years, this was not statistically significant ($p=.7$). The BMD was found to be significantly associated with body mass index (BMI) ($p\leq.0001$) at both measurement sites, with an increase of one unit of BMI translating to an increase of 0.0044 g/cm^2 in radius BMD. Follicle-stimulating hormone (FSH) level $\geq 25.8\text{ mIU/mL}$ was associated with a decrease of 0.017 g/cm^2 in radius BMD relative to women with FSH $<25.8\text{ mIU/mL}$. Significant interaction between FSH and BMI in their effect on BMD was observed ($p=.006$). This study found no evidence that long-term use of DMPA, NET-EN and COCs affects BMD in this population.

© 2005 Elsevier Inc. All rights reserved.

Keywords: Depot-medroxyprogesterone acetate; Norethisterone enanthate; Combined oral contraceptives; Bone mineral density

1. Introduction

Cross-sectional studies undertaken to investigate the effect of hormonal contraceptives on bone mineral density (BMD) have produced conflicting reports [1–7]. Most have indicated that depot-medroxyprogesterone acetate (DMPA) has a negative effect on bone mass [1–5], and some have shown minimal or no effect [6–8]. The loss of BMD in the case of DMPA has been associated with estrogen deficiency [9]. Some of these studies show that these negative changes are reversible and there is a return to pre-use levels on

discontinuation of the method [1,10]. In addition, there has been evidence of recovery of BMD in long-term users [1,6,11]. There is no information available on the effect of norethisterone enanthate (NET-EN) on bone mass; however, published studies on norethisterone (NET) have shown a positive effect on BMD [12–14]. Combined oral contraceptives (COCs) have not been found to be associated with loss of bone mass [15,16], with some studies indicating that COCs are bone-sparing [16,17].

Few studies have investigated the effect of hormonal contraceptives on BMD in premenopausal long-term users of hormonal methods in their 40s, and none have investigated the effect of NET-EN on BMD.

Depot-medroxyprogesterone acetate and norethisterone enanthate are effective contraceptives with low failure rates

* Corresponding author. Tel.: +27 31 304 8383; fax: +27 31 304 8468.
E-mail address: m.beksinska@thru.co.za (M.E. Beksinska).

[18]. In South Africa, hormonal injectable contraceptive use is high, with about 60% of Black African women practicing contraception using either DMPA or NET-EN [19]. Of the two available injections, older women mainly use DMPA and often continue to use this method into their late 40s. Use of COCs is lower with the method predominantly used by white and Asian women. As menopause is known to be associated with bone loss [20], it would be important to know if hormonal methods of contraception could further contribute to bone loss in older women.

The aim of this study was to determine if long-term use of hormonal contraceptives (COCs, DMPA and NET-EN) is associated with change in bone mass in women aged 40–49 years.

2. Subjects and methods

A cohort of women 40–49 years old using DMPA, NET-EN or COCs and nonusers of hormonal contraception were recruited from a large family planning clinic in Durban, South Africa. Recruitment commenced in 2000 and was completed in 2003. For inclusion as a hormonal contraceptive user, women had to have used DMPA, NET-EN or COCs for at least 1 year. For inclusion in the nonuser control group, women should not have used any form of hormonal contraception in the past year. All DMPA users were on a regimen of 150 mg every 12 weeks and NET-EN users on 200 mg every 8 weeks. The COCs used by women included a range of formulations, with most (93%) using

formulations containing between 30 and 40 µg of estrogen. Women were eligible to participate if they had not lactated or delivered in the past 6 months, were not currently or had never used medication known to affect calcium metabolism for more than 3 months and did not have a chronic disease affecting calcium metabolism. Women who were postmenopausal were excluded from the study at screening as the aim of the study was to follow-up women through their natural menopause. Menopause was classified as amenorrhea for at least 1 year. For injectable hormonal contraceptive users this criterion could not always be used as women commonly experienced amenorrhea induced by the method. Therefore, in addition to amenorrhea, follicle-stimulating hormone (FSH) levels from blood samples were measured. An FSH level of ≥ 25.8 mIU/mL was considered to be in the menopausal range (King Edward VIII Hospital Durban; Chemical Pathology Laboratory criteria using Roche Elecsys FSH expected values).

On recruitment, a questionnaire was administered by a trained research nurse to elicit information on lifetime contraceptive history, fertility history, menopausal symptoms and regularity of the menstrual cycle. Questions were also asked on smoking, diet, exercise and caffeine and alcohol intake. The examination included height, weight, blood pressure and waist and hip measurements using a standard protocol. Body mass index (BMI) was calculated from the weight and height as kg/m^2 . Forearm BMD was measured by dual energy X-ray absorptiometry (DEXA model DTX-200; Osteometer MediTech, Rodovre, Den-

Table 1
Sociodemographic and lifestyle characteristics of subjects in the 40 to 49 year age range by contraceptive method use

Characteristics	DMPA (<i>n</i> = 127)	NET-EN (<i>n</i> = 102)	COC (<i>n</i> = 106)	Nonuser (<i>n</i> = 161)	p value
Mean age, years (SD)	43.6 (2.7)	43.0 (2.2)	43.7 (2.5)	45.4 (2.5)	<.0001
Mean highest education grade (SD)	8.5 (3.3)	9.8 (3.1)	9.8 (2.4)	9.1 (2.7)	.012
Marital status (%)					<.0001
Married/cohabiting	46.1	52.1	57.1	41.0	
Regular partner-not cohabiting	3.2	7.7	5.7	3.7	
Casual partner	38.9	36.5	28.6	25.5	
No partner	11.9	2.9	7.6	29.8	
Employment status (%)					<.0001
Employed full/part-time	44.5	73.5	71.5	63.4	
Unemployed	54.0	19.6	23.8	33.5	
Housewife	1.6	6.9	4.8	3.1	
Ethnicity (%)					.007
African	98.4	95.2	67.0	94.4	
Colored	1.6	1.0	7.5	2.5	
Indian	0	3.8	25.5	3.1	
Exercise					.48
No regular exercise (%)	96.9	96.0	94.3	93.0	
At least once a week (%)	3.1	4.0	5.7	7.0	
Dieted in last 6 months (%)	0	0	4.7	<1	.003
Caffeine consumption ^a					.08
Never (%)	9.1	18.8	19.0	12.3	
1–2 cups/day (%)	75.3	62.2	69.1	75.2	
>2 cups (%)	15.6	19.0	12.0	12.6	
Ever smoked cigarettes (%)	11.0	10.3	10.4	16.1	.17
Current smoker (%)	4.7	5.6	5.7	9.9	.23

^a Tea or coffee.

Table 2

Reproductive characteristics by contraceptive method user group

Characteristics	DMPA (<i>n</i> = 127)	NET-EN (<i>n</i> = 104)	COC (<i>n</i> = 106)	Nonuser (<i>n</i> = 161)	p value
Parity (median)	4	3	3	3	.04
Ever lactated (%)	88.2	91.3	87.7	83.2	.34
Mean age at menarche, years (SD)	15.2 (1.7)	15.5 (1.7)	14.8 (1.7)	14.8 (1.7)	.014
Lactation (years), median	3.5	3.2	3.0	3.2	.06
FSH					<.0001
<25.8 mIU/mL (%)	72	94	66	90	
>25.8 mIU/mL (%)	28	6	34	10	
Use of group method ^a					
Median use in last 5 years (months)	53	45	52	NA	
Median lifetime use (months)	84	49	89	NA	
Mean age at first use (years)	36	37	36	NA	

^a Only group method shown, that is, group to which women using that method at the time of recruitment were allocated. Contraceptive history of nonuser group not shown.

mark). BMD was measured in g/cm² at two sites in the distal forearm (radius and ulna). The distal site contains approximately 25% trabecular bone and 75% cortical bone. The DEXA equipment was standardized daily using a phantom as prescribed by the manufacturer's instructions. Accuracy to the standard during the recruitment period was 0.53% and in vivo precision was 0.94%. Study participants are currently being followed-up at six monthly intervals for a total of 5 years. Women will be completing their 5-year follow-up visit between 2005 and 2008. The findings presented in this paper are based on the baseline data collected at the recruitment visit and are cross-sectional in nature.

The characteristics of women in the study were quantified as means $\pm$ SD, medians or percentages. Follicle-stimulating hormone levels were divided into two categories according to laboratory cut-off levels for premenopausal (<25.8 mIU/mL) and perimenopausal/menopausal ($\geq$ 25.8 mIU/mL). Differences in BMD between contraceptive groups and the associations between BMD and selected characteristics of the study participants by contraceptive group were assessed using one-way analysis of variance and multiple variable linear regression for BMD measured at the two anatomic sites. The proposed study aims to be able to detect a half standard deviation difference in bone mass between users and nonusers of injectable contraceptives. This would be of biological significance as this difference would translate into a large difference in the risk of fracture in the older woman. Information on the mean and standard deviation of cross-sectional measurements of forearm bone mass in white, European, premenopausal women reported by Nordin [21] was used to estimate sample size. The sample size, calculated assuming a two-tailed statistical test with a significance level of 5% and with power of 80%, was 63 subjects in each contraceptive group. Loss to follow-up was estimated at approximately 10–15% per year. Therefore, sample size after adjusting for loss to follow-up was at least 100 women in each category.

Data were analyzed using the statistical package STATA (V.8; College Station, TX, USA). Ethical approval was

granted by the University of the Witwatersrand, Human Subjects Research Committee, and by the Scientific and Ethical Review Group of the World Health Organization.

3. Results

In total, 496 women were recruited. Baseline information about these women is summarized in Table 1. Although the groups using hormonal contraception were similar in age, the nonusers of hormonal contraception were on average 2 years older than DMPA users ($p < .001$). Norethisterone enanthate users were the youngest group with a mean age of 43 years. Almost all women were African except for the COC group which included around a quarter (26%) of Indian women. This resulted in a significant difference in ethnicity between the groups ($p = .007$). The reproductive characteristics of the woman are shown in Table 2. A number of other differences were noted between the groups including a significant difference in age of menarche, FSH, parity, employment status, marital status and education. There were no differences in smoking history, exercise (Table 1) or years of lactation (Table 2).

All women using hormonal methods had used the method for at least 1 year at recruitment. The average length

Table 3
Factors potentially associated with radius BMD (unadjusted)

Factors	Radius BMD g/cm ² (95% CI)	p value
Contraceptive group		.26
DMPA	0.514 (0.501–0.527)	
NET-EN	0.514 (0.499–0.528)	
COC	0.500 (0.486–0.514)	
Nonuser	0.518 (0.506–0.529)	
Ethnicity		.007
African	0.516 (0.509–0.523)	
Indian	0.491 (0.451–0.531)	
Colored	0.479 (0.456–0.503)	
Age, per year	–0.0017 (–0.0041 to 0.0008)	.188
BMI, change per kg/m ²	0.0044 (0.0036–0.0052)	<.001
FSH		.029
<25.8 mIU/mL (%)	0.516 (0.508–0.524)	
>25.8 mIU/mL (%)	0.498 (0.483–0.512)	

Table 4
Results of multiple regression model with BMI and FSH level

Factors	Radius BMD units (95% CI)	p value
Effect of FSH ^a (for women of median BMI=33.9 kg/m ²)		
<25.8 mIU/mL	0.514 (0.507–0.521)	<.066
>25.8 mIU/mL	0.501 (0.488–0.513)	
Effect of BMI, unit BMD per unit change in BMI		<.001
FSH<25.8 mIU/mL	0.0038 (0.0028–0.0047)	
FSH>25.8 mIU/mL	0.0067 (0.0048–0.0086)	

^a Significant interaction between FSH level and BMI ($p=.006$).

of lifetime use for DMPA was 9.3 years with a number of women ($n=13$) having used the method for 15 years or more (Table 2). Norethisterone enanthate users had been using the method on average for 5.6 years and COC users for 8.6 years.

There was no significant difference in BMD between the four contraceptive user groups at the radius ($p=.26$) or the ulna site ($p=.21$), with and without adjustment for age (Table 3). Although a small decrease in BMD was noted over the age range (40–49 years), this was not statistically significant ($p=.7$). Length of use of method in the last 5 years and total lifetime use were not associated with difference in BMD.

Although differences were noted between the contraceptive groups, most were not associated with BMD except for BMI, ethnic group and FSH level (Table 3). A statistically significant difference in BMD was found between the Indian and African women ($p=.014$); however, after adjusting for BMI, the difference in BMD was no longer significant.

Bone mineral density was found to be associated with BMI ($p\leq.0001$) and with FSH level at the radius ($p=.029$) and ulna ($p=.022$). An increase of one unit of BMI was associated with an increase of 0.0044 g/cm^2 in radius and 0.0041 g/cm^2 in ulna BMD. Follicle-stimulating hormone level $>25.8\text{ mIU/mL}$ was associated with a decrease of 0.017 g/cm^2 in radius BMD (Table 3) relative to women with FSH $<25.8\text{ mIU/mL}$.

Body mass index, FSH level (≥ 25.8 vs. $<25.8\text{ mIU/mL}$) and interaction of BMD with FSH level were all significantly associated with BMD in a multiple regression model ($r^2=.2$). According to this model, for women of median BMI of 33.9 units, mean radius BMD varied from 0.514 g/cm^2 (95% CI, $0.507\text{--}0.521$) with FSH $<25.8\text{ mIU/mL}$, to 0.501 g/cm^2 (95% CI, $0.488\text{--}0.513$) for women with FSH $>25.8\text{ mIU/mL}$ ($p=.066$). The effect of FSH on BMD was significantly modified by BMI and vice versa ($p=.006$). For women with FSH $<25.8\text{ mIU/mL}$, BMD increased by 0.0038 (95% CI, $0.0028\text{--}0.0047$) (g/cm^2) per unit increase in BMI, whereas for women with FSH $>25.8\text{ mIU/mL}$, BMD increased by 0.0067 g/cm^2 (95% CI, $0.0048\text{--}0.0086$) for each unit

increase in BMI (Table 4). Similar results were obtained for ulna BMD (data not shown).

Follicle-stimulating hormone level was found to be significantly different between user groups ($p\leq.0001$). However, after adjusting for age, the difference between the four groups was no longer significant ($p=.13$). An increase of 1 year in age increased mean FSH level by 3 mIU/mL ($p<.001$).

4. Discussion

The findings of this cross-sectional study of older women (40–49 years) show no evidence of a significant difference in BMD between the three contraceptive users and nonuser control groups. This is in agreement with some studies that have found no difference in BMD in long-term users of DMPA compared to nonuser controls [1,6] and the normal population mean [8]. Some of these studies [1,8] suggest that any bone loss would occur in approximately the first 3 years of use but that this effect could be reversible. However, other studies have shown that loss of BMD persists in long-term users [22,23]. The mechanism involved in reversing a hormonally initiated loss of BMD is not clear. It has been suggested [1] that a homeostatic mechanism operates that reverses bone loss after prolonged hormonal stimulation. Women using contraceptives in the study reported here were mainly long-term users and it may be that any initial loss of BMD associated with use of DMPA had been recovered.

While this study did not show that COC use was beneficial with respect to bone mass, some studies have shown a positive association of BMD with COCs [6,17]. It could be speculated that a short-term increase in BMD as a result of COC use may return to the BMD levels of nonusers after some time. In support of this, several studies have found that there is no difference in BMD in long-term users of COCs [1,24].

This is the first study to include data on the two-monthly injectable NET-EN. It is suggested that NET may protect against calcium loss via a direct topic effect on bone or by the action of estrogenic metabolites [25]. Norethisterone enanthate can be considered to be similar in action to NET, as the enanthate (EN) is added primarily to give it its depot characteristics. Although the studies that have included NET [12–14] have shown a positive effect on BMD, no positive effect of NET-EN was seen compared to other groups in this study.

No previous studies have reported on the relationship between FSH level and BMD which may be more important than age, as raised FSH is an indicator for menopause. In our study, women with an FSH level of $\geq 25.8\text{ mIU/mL}$ had a significantly lower BMD at both distal sites. We have found a significant interaction between BMI and FSH in their effect on BMD. As a result of this, the loss in BMD for an increase in FSH level $>25.8\text{ mIU/mL}$ will be greater for women of lower BMI than for their counterparts with relatively higher

BMI. For example, a woman with BMI at the 25th percentile of BMI in our sample (28.9 units) will lose 0.0285 g/cm² (95% CI, –0.0462 to –0.0108) of BMD when moving from the lower category of FSH. By contrast, a woman at the 75th percentile of BMI (38.54 units) will lose only 0.0003 g/cm² (95% CI, –0.018 to 0.0175) of BMD when her FSH level increases to more than 25.8 mIU/mL according to this model. In other words, loss in BMD as a result of menopause is diminished by the effect of BMI.

In women with FSH 25.8 IU/mL, BMD increased by 0.0067 g/cm² per unit increase of BMI, whereas in women with FSH levels <25.8 mIU/mL, BMD increased by 0.0038 g/cm² per unit increase in BMI. This means that the effect of BMI on bone density is greater for women with raised FSH levels, compared to women with FSH <25.8 mIU/mL. Using the cut-off of FSH of 25.8 mIU/mL as a marker of menopause, the protective effect of BMI is greater for postmenopausal women (FSH >25.8 mIU/mL) than it is for premenopausal women (FSH <25.8 mIU/mL).

Investigation of older women was considered important as any loss of BMD during short-term use of DMPA could be exacerbated by age. There is, however, some debate around the extent of age-related bone loss [20,26]. There was a small nonsignificant difference in BMD with age noted in the population in our study. Since the age range of our participants was narrow (10 years) a significant difference in BMD and age may not have been expected.

This study confirms the association of BMD with BMI [1,11,23]. In our study, African women had a significantly higher BMI and BMD compared to the Indian women. Petitti et al. [1] found that the Black African women in Harare had a higher mean BMI and that ethnic differences in BMD persisted even after adjustment for BMI. However, in our study, differences in BMI explained the ethnic differences in BMD.

In conclusion, long-term use of hormonal methods in this study population does not negatively impact on BMD and there should not be concerns about allowing women to continue using this method until they reach menopause. Further, since African women have generally higher BMIs than other population groups, their risk of osteoporosis may be lower.

4.1. Limitations of the study

The cross-sectional nature of the study is a limitation. The women recruited are now contributing to a longitudinal study with 5 years of follow-up. Bone mineral density measurements were limited to the forearm and, therefore, findings may not be applicable to other skeletal sites.

Acknowledgments

This study was supported by a grant from the World Health Organization and the Department for International Development UK (DFID).

References

- [1] Petitti DB, Piaggio G, Menta S, Cravioto MC, Meirik O. Steroid hormone contraception and bone mineral density: a cross-sectional study in an international population. *J Obstet Gynaecol* 2000;95:736–43.
- [2] Scholes D, Lacroix AZ, Ott SM, Ichikawa LE, Barlow WE. Bone mineral density in women using depot medroxyprogesterone acetate for contraception. *J Obstet Gynaecol* 1999;93:233–8.
- [3] Wanichsetakul P, Kamudhamas A, Watanaruagkovit P, Siripakam Y, Visutakul P. Bone mineral density at various anatomic bone sites in women receiving combined oral contraceptives and depot-medroxyprogesterone acetate for contraception. *Contraception* 2002; 65:407–10.
- [4] Cundy T, Farquhar CM, Cornish J, Reid IR. Short-term effects of high dose oral medroxyprogesterone acetate on bone density in premenopausal women. *J Clin Endocrinol Metab* 1996;81:1014–7.
- [5] Cundy T, Evans M, Roberts H, Waltie D, Ames R, Reid IR. Bone density in women receiving depot medroxyprogesterone acetate for contraception. *BMJ* 1991;303:13–6.
- [6] Taneepanichskul S, Intaraprasert S, Theppisai U, Chaturachirda K. Bone mineral density in long-term depot-medroxyprogesterone acetate acceptors. *Contraception* 1997;56:153–5.
- [7] Bahamondes L, Perotti M, Castro S, Faundes D, Petta C, Bedone A. Forearm bone density in users of Depo-Provera as a contraceptive method. *Fertil Steril* 1999;71:849–52.
- [8] Gbolade B, Ellis S, Murby B, Randall S, Kirkman R. Bone density amongst long-term users of medroxyprogesterone acetate. *Br J Obstet Gynaecol* 1998;105:790–4.
- [9] Ortiz A, Hiroi M, Stanczyk FZ, Goebelsmann U, Mishell DR. Serum medroxyprogesterone acetate (MPA) concentrations and ovarian function following intramuscular injection of Depo-MPA. *J Clin Endocrinol Metab* 1977;44:32–8.
- [10] Orr-Walker BJ, Evans MC, Ames RW, Clearwater JM, Cundy T, Reid IR. The effect of past use of the injectable contraceptive depot medroxyprogesterone acetate on bone mineral density in normal postmenopausal women. *Clin Endocrinol* 1998;49:615–8.
- [11] Tang OS, Tang G, Yip PSF, Li B. Further evaluation of long-term depot-medroxyprogesterone acetate use and bone mineral density; a longitudinal cohort study. *Contraception* 2000;62:161–4.
- [12] Eldred JM, Haynes PJ, Thomas EJ. A randomized double blind placebo controlled trial of the effects on bone metabolism of the combination of nafarelin acetate and norethisterone. *Clin Endocrinol* 1992;37:354–9.
- [13] Horowitz M, Wishart JM, Need AG, Morris HA, Nordin BEC. Effects of norethisterone on bone related biochemical variables and forearm bone mineral in postmenopausal osteoporosis. *Clin Endocrinol* 1993;39:649–55.
- [14] Riis BJ, Christiansen C, Johansen JS, Jacobson J. Is it possible to prevent bone loss in young women treated with luteinizing hormone-releasing hormone agonists? *J Clin Endocrinol Metab* 1990;70:920–4.
- [15] De Cherney A. Bone-sparing properties of oral contraceptives. *Am J Obstet Gynecol* 1996;174:15–20.
- [16] Menta S. Bone loss, contraception and lactation. *Acta Obstet Gynecol Scand* 1993;72:148–56.
- [17] Kleerkoper M, Brienza RS, Schultz LR, et al. Oral contraceptive use may protect against low bone mass: Henry Ford Hospital Osteoporosis Cooperative Research Group. *Arch Intern Med* 1991;151:1971–6.
- [18] World Health Organization Special Programme of Research, Development and Research Training in Human Reproduction. Task Force on Long-Acting Systemic Agents for the Regulation of Fertility. Multinational comparative clinical evaluation of two long-acting injectable contraceptive steroids: norethisterone enanthate given in two dosage regimens and depot medroxyprogesterone acetate: final report. *Contraception* 1983;23:1–20.
- [19] Department of Health South Africa, Medical Research Council and Measure DHS (2002). South African demographic and health survey 1998, full report. Pretoria (South Africa): Department of Health; 2002.

- [20] Ahlborg MG, Johnell O, Nilsson BE, Jeppsson S, Ronnevik G, Karlson MK. Bone loss in relation to menopause: a prospective study during 16 years. *Bone* 2001;28:327–31.
- [21] Nordin BEC. The definition and diagnosis of osteoporosis. *Calcif Tissue Int* 1987;40:57–8.
- [22] Cundy T, Cornish J, Roberts H, Reid IR. Menopausal bone loss in long-term users of depot medroxyprogesterone acetate contraception. *Am J Obstet Gynecol* 2002;186:978–83.
- [23] Pavia LC, Pinto-Neto AM, Faundes A. Bone density among long-term users of medroxyprogesterone acetate and bone mineral density. *Contraception* 1999;59:25–9.
- [24] Perrotti M, Bahamondes L, Petta C, Castro S. Forearm bone density in long-term users of oral combined contraceptives and depot medroxyprogesterone acetate. *Fertil Steril* 2001;76:469–73.
- [25] Christiansen C, Riis BJ, Nilas L, Rodbri P, Deftos L. Uncoupling of bone formation and resorption by combined oestrogen and progestagen therapy in postmenopausal osteoporosis. *Lancet* 1985;2:800–1.
- [26] Sowers MF, Galuska DA. Epidemiology of bone mass in premenopausal women. *Epidemiol Rev* 1993;15:374–98.

Detection of raised FSH levels among older women using depomedroxyprogesterone acetate and norethisterone enanthate

Mags E. Beksinska^{a,*}, Jenni A Smit^a, Immo Kleinschmidt^b, Helen V. Rees^a,
Tim M.M. Farley^c, Franco Guidozi^d

^a*The Reproductive Health Research Unit, Department of Obstetrics and Gynaecology, University of the Witwatersrand, Suite 1301, Maritime House, 143 Salmon Grove, Durban, South Africa*

^b*Family Health International, P.O. Box 13950, Research Triangle Park, NC 27709, USA*

^c*Special Programme of Research, Development and Research Training in Human Reproduction, Department of Reproductive Health and Research World Health Organisation, Avenue Appia 20, 1211 Geneva 27, Switzerland*

^d*Department of Obstetrics and Gynaecology, Johannesburg Hospital, University of the Witwatersrand, Johannesburg, South Africa*

Received 31 March 2003; received in revised form 31 July 2003; accepted 6 August 2003

Abstract

The objective of this study was to investigate whether follicle-stimulating hormone (FSH) levels can be used reliably to indicate approaching menopause in older (aged 40–49), long-term users of depomedroxyprogesterone acetate (DMPA) and norethisterone enanthate (NET-EN). One-hundred and seventeen women using DMPA, 60 NET-EN users and 161 nonusers of contraception were recruited. At recruitment, serum FSH levels were measured and questions were asked regarding menopausal symptoms, menstrual cycle and date of last injection. Results of the recruitment blood test showed that 32% of the nonusers had FSH levels in the menopausal range >25.8 mIU/mL compared to 28% of the DMPA users and 9% of the NET-EN group. After adjusting for age, there was no significant difference between the 3 groups ($p = 0.13$). An increase of 1 year in age increased the FSH level by 3 mIU/mL ($p < 0.001$). All the hormonal contraceptive users were between 1 day and 12 weeks of their injection interval. Many had been using the injectable contraceptive method for over 10 years and almost all were amenorrheic at the time of recruitment. The data show that a raised FSH level can be detected during use of DMPA and NET-EN and could be used as a menopausal indicator without interrupting method use in this group of contraceptive users. © 2003 Elsevier Inc. All rights reserved.

Keywords: Depomedroxyprogesterone acetate; Norethisterone enanthate; Menopause; Follicle-stimulating hormone

1. Introduction

Depomedroxyprogesterone acetate (DMPA) and norethisterone enanthate (NET-EN) are effective contraceptives with low failure rates [1]. One of the common side effects of these methods is amenorrhea, which increases in incidence over time [1]. In older users it may be difficult to distinguish between amenorrhea associated with approaching menopause and that induced by the method. Detection of menopause or perimenopause, therefore, presents a challenge in this group of contraceptive users. Traditional textbook management of an older woman using oral hormonal contraception recommends measurement of follicle-stimulating hormone (FSH) on the 7th day of the pill-free interval [2]. Advice for DMPA and NET-EN users is less clear; however, in the absence of data documenting efficacy, it has been suggested that they should change to a nonhormonal method of contraception, as menopausal symptoms will be masked, and measurement of FSH levels may be unreliable [2].

DMPA and NET-EN are known to inhibit the mid-cycle surge of FSH and luteinizing hormone (LH), but the tonic release of these gonadotrophins continues at luteal phase levels [3,4]. A few studies have also shown that DMPA and NET-EN suppress raised FSH and LH levels in postmenopausal women [5,6]. While limited information exists on the duration of the suppression of raised FSH and LH levels associated with menopause, in both these studies [5,6], the lowest FSH and LH levels occurred between 14 and 30 days after injection. The majority of suppressed levels were still

DMPA and NET-EN are known to inhibit the mid-cycle surge of FSH and luteinizing hormone (LH), but the tonic release of these gonadotrophins continues at luteal phase levels [3,4]. A few studies have also shown that DMPA and NET-EN suppress raised FSH and LH levels in postmenopausal women [5,6]. While limited information exists on the duration of the suppression of raised FSH and LH levels associated with menopause, in both these studies [5,6], the lowest FSH and LH levels occurred between 14 and 30 days after injection. The majority of suppressed levels were still

* Corresponding author. Tel.: +31-304-8383; fax: +31-304-8468.
E-mail address: m.beksinska@rhu.co.za (M.E. Beksinska).

high enough to be classified as in the menopausal range. Thereafter, FSH and LH levels started to rise, and in one of these studies [6], FSH levels started rising back to presuppression levels between 40 and 80 days after injection.

In South Africa, hormonal injectable contraceptive use is high, with about 60% of Black African women practicing contraception using either DMPA or NET-EN [7]. Of the two available injections, older women almost exclusively use DMPA and often continue to use this method into their late 40s.

While the primary aim of this study was to investigate bone density in older women using hormonal contraceptives, a secondary objective was to investigate whether FSH levels could be used as an indicator of menopausal status in injectable users. This was due to the high prevalence of amenorrhea in the two injectable groups, which made it impossible to use the menstrual cycle as an indicator of menopausal status. This paper presents the results of the latter component of the study.

2. Materials and methods

A cohort of older women using DMPA (150 mg at 3-month intervals), NET-EN (200 mg at 2-month intervals), combined oral contraceptives (COCs) and nonusers of contraception were recruited from a large family-planning clinic in Durban, South Africa, between September 2000 and November 2001. For inclusion as an injectable user, women had to be aged between 40 and 49 years and had to have used the injectable hormonal contraceptive for at least 1 year. On recruitment, a questionnaire was administered that elicited information on contraceptive history, menopausal symptoms and regularity of the menstrual cycle. Because women were recruited at different times in the injection cycle, date of last injection was recorded. A subsample of injectable users with FSH levels >25.8 mIU/mL at recruitment were contacted and asked to return for a second test to confirm their earlier result. If the recruitment measurement was taken later in the injection cycle, the woman was asked to return for a repeat test in the expected peak suppression period in her next injection cycle (14–30 days). If the recruitment measurement was taken during time of expected peak suppression she was asked to return after this time in the next injection cycle. FSH measurements are repeated yearly for the 5-year duration of the study.

A serum sample was obtained at the recruitment visit, and serum FSH levels were determined by immunoassay on the Roche Modular Analytics E170 (Elecsys module) immunoassay analyzer. Repeated samples from each subject were always analyzed in the same FSH assay batch. In total, eight assays were conducted during the time of data collection for this study. For the purposes of this study, an FSH level between 25.8 and 135 mIU/mL was considered to fall into the menopausal range (King Edward VIII Hospital

Durban; Chemical Pathology Laboratory criteria using Roche Elecsys FSH expected values).

2.1. Assay specificity

With respect to analytical specificity, the following cross reactions with other hormones have been determined for the monoclonal antibody used: LH, thyroid-stimulating hormone, human chorionic gonadotropin, human growth hormone and human placental lactogen at $<0.1\%$.

2.2. Assay sensitivity

The lower detection limit is <0.10 mIU/mL. The detection limit represents the lowest FSH level that can be distinguished from zero. It is calculated as the concentration lying two standard deviations above that of the lowest standard. The maximum of the measuring range is 200 mIU/mL. Values above this figure are reported as >200 mIU/mL. All samples analyzed for this study were above the lower detection limit and no sample was reported over 200 mIU/mL.

2.3. Test precision (reproducibility)

The within-assay coefficients of variation ranged between 0.78% and 1.5% for QCs with mean values of 9.53 and 33. Between assay variability ranged from 3.5% to 7.6%, depending on the serum FSH concentration.

To investigate whether DMPA and NET-EN interfered with the FSH assay, control test samples and patient samples with high and low values of FSH were spiked with both contraceptives at levels above the highest circulating levels of MPA and NET described in the literature [8,9]. The results of this showed that only very small differences were observed, which fell into the quoted within-run precision of the test.

In this article we present the data collected up to November 2001 relating to the injectable hormonal users. Data on COC users will be analyzed separately. Mean and median FSH levels were compared between nonusers and each of the two injectable user groups, by means of Student's *t* test and the Wilcoxon test, respectively, using the statistical package STATA (V.7 College Station, TX, USA). Multiple regression was done to investigate the effect of hormonal injectable use on FSH, adjusted for age. Ethical approval was granted by the University of the Witwatersrand, Human Subjects Research Committee.

3. Results

In total, 117 DMPA and 60 NET-EN users were recruited. Fewer women were recruited in the NET-EN group, as this method is not as popular in older women. Baseline information about these women and 161 nonusers of con-

Table 1
Baseline information

	Nonusers (n = 161)	DMPA users (n = 117)	NET-EN users (n = 60)
Mean Age (y) ($p < 0.001$)	45.3	43.6	42.5
Median	46	43	42
Range	40–49	40–49	40–49
Mean FSH mIU/mL ($p = 0.013$)	26.4	22.1	11.6
Median ($p = 0.013$)	12.6	10.1	7.2
Range	0.2–115	0.1–114	0.2–51.1

contraception is summarized in Table 1. Nonusers of hormonal contraception were on average 2 years older than DMPA users ($p < 0.001$). NET-EN users were the youngest group with a mean age of 42.5 years. All women using either DMPA or NET-EN had been using the method for at least 1 year at recruitment. The average length of lifetime use for DMPA was 9.3 years, with a number of women ($n = 13$) who had used the method for 15 years or more. NET-EN users had been using the method on average for 5.6 years. The majority of subjects had started using DMPA or NET-EN immediately after the birth of their last child, as they had attained their desired family size. Very few women using DMPA reported regular menstrual cycles (8%); women not reporting a regular cycle were in almost all cases experiencing spotting or amenorrhea. More women in the NET-EN group (48%) reported regular cycles. Results of the recruitment blood test showed that 32% of the nonusers had FSH levels in the menopausal range (>25.8 mIU/mL) compared to 28% of the DMPA users and 9% of the NET-EN group. FSH was associated with a woman's age (p

Table 2

Individual FSH levels of the subgroup of users of DMPA and NET-EN with raised FSH levels at recruitment

Subject no.	Days postinjection (earlier measurement)	FSH (mIU/mL)	Days Postinjection (later measurement)	FSH (mIU/mL)
DMPA				
391	50	52.9	85	71.8
584	42	38.7	48	37.4
628	27	28.6	37	57.9
629	14	113	42	99.7
632	14	22.9	90	45.6
703	25	9.3	72	93.3
706	76	32.7	90	45.1
734	55	54.3	93	68.2
740	52	54.6	91	50.5
792	17	67.9	36	69.4
822	5	30.8	14	33.4
833	1	50.2	28	41.5
NET-EN				
639	15	21.3	38	28.5
724	10	27.4	60	51.2

$= 0.013$), and Fig. 1 shows a plot of FSH levels by age predicted from the regression model. After adjusting for age, there was no significant difference in FSH levels among the three groups ($p = 0.13$). An increase of 1 year in age increased the FSH levels by 3 mIU/mL ($p < 0.001$).

Of the total 33 women using DMPA and 6 using NET-EN who had an FSH level greater than 25.8 mIU/mL on their recruitment visit, 12 DMPA and 2 NET-EN users were able to return during their next injection cycle after being contacted by telephone. Table 2 shows the two results

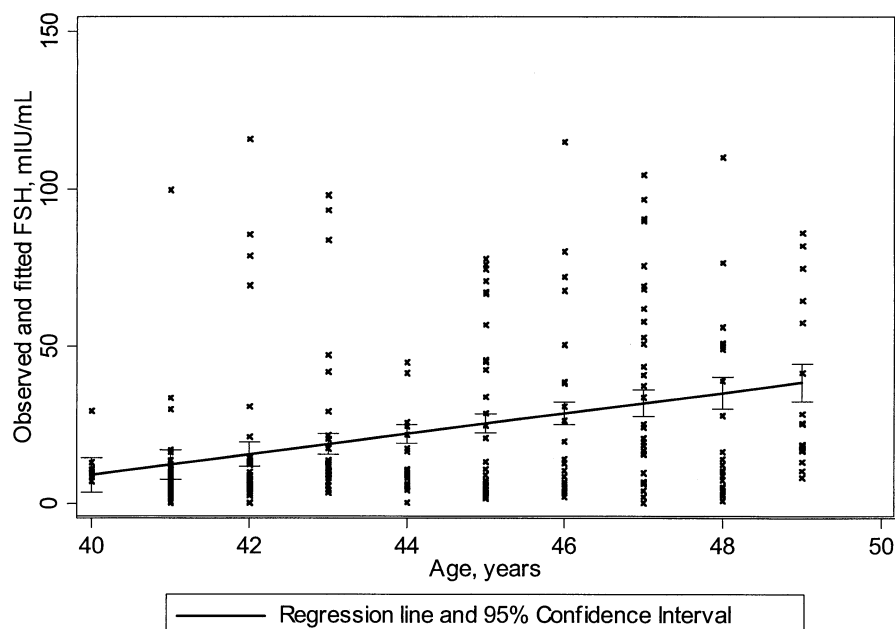


Fig. 1. Observed and fitted FSH levels by age.

in each of these participants. In total, 7 women had one of the two FSH measurements taken in the time of expected peak suppression (14–30 days). In three of these measurements the FSH level fell below the menopausal range (<25.8 mIU/mL) while the other four still remained in the menopausal range (>25.8 mIU/mL). The levels in most cases differed considerably depending on the time postinjection. The level taken later in the injection cycle was higher in 10 of the 14 cases. In two subjects (subject nos. 629 and 792), similar FSH levels were observed during expected peak suppression (14–30 days) and later in the injection cycle.

4. Discussion

Previous studies have been limited due to their small sample sizes and the inclusion of subjects who received only one dose of MPA or NET-EN [5,6]. These studies concluded that the baseline values of FSH and LH in postmenopausal women declined from the day of injection to reach their lowest levels between 14 and 30 days and then rose back to presuppression levels between about 40 and 80 days postinjection. In one of these studies [5], one of the subjects received a second dose of NET-EN and results indicated that the suppression of FSH was not as pronounced as that of the first dose. Further, the degree of suppression of FSH is reported to vary considerably between women, with suppression ranging between 30% and 75% of baseline values [5]. Although a great deal of variability between women was shown, a similar pattern of suppression after injection and return to baseline values later in the dosing cycle was exhibited [5].

Our study supports the earlier work, in that in almost all cases, DMPA and NET-EN did suppress FSH levels in the first 30 days after injection. Where two samples were taken in the same subject, the levels of FSH measured in the sample taken sooner postinjection were lower in almost all cases. In two cases, the gonadotrophin levels were measured on day 1 (subject no. 833) and day 5 (subject no. 822) postinjection, probably before peak suppression occurred. In one case (subject no. 833) the level taken subsequently at day 28 during the time of expected peak suppression was found to be lower, while in the other (subject no. 822) the level was slightly higher. It may be that in this subject the FSH level was already suppressed by day 5 and by day 14 it was recovering.

In some subjects, both measurements were taken after the time of expected peak suppression and in these cases the differences between the two measurements was smaller; however, the measurement taken later in the cycle was similar or higher. The subject with the highest FSH level (subject no. 629) showed little difference between her level at 13 days (113 mIU/mL) and that taken at 42 days (99.7 mIU/mL). It may be that the peak suppression of FSH

occurred between these two times or that gonadotrophin levels in this subject only responded marginally in terms of suppression. This subject had been using the method for 14 years in total.

This is the first study which examines FSH levels in long-term users of DMPA and NET-EN. Raised FSH levels were found in women during use of the method and this indicates that measurement of gonadotrophins could be used as an indicator of onset of menopause. This also supports earlier work where gonadotrophin levels rarely fell below a peak level of 20 mIU/mL in postmenopausal women [5,6], again suggesting that the suppression would not necessarily make gonadotrophin levels unreliable indicators of menopause in hormonal injection users. It does suggest, however, that the measurement should be taken as late as possible in the 2- or 3-month injection cycle to ensure that the gonadotrophin levels were approaching baseline levels.

There are no local guidelines in South Africa on how to deal with a woman using injectable contraception in her later reproductive years; however, the policy at a large provincial hospital in Durban is to refer users reaching 50 years, or users who report menopausal symptoms, for FSH measurement at the end of a 3-month DMPA injection cycle (King Edward Hospital, Durban, personal communication). If an FSH level is found to be in the menopausal range, the woman will be counseled to consider changing her method of contraception to a barrier method to see if menses returns. Raised levels of FSH with accompanying menopausal symptoms have been documented at this facility amongst hormonal injection users. This finding has important implications for guiding family-planning providers in the management of older women using hormonal injectable contraception. Women could possibly have gonadotrophin levels measured without discontinuing the method, as long as the test is taken later in the injection cycle for both NET-EN and DMPA. A good time to measure levels is on return for the next injection dose before the gonadotrophin levels are reduced.

The transition to menopause described as the perimenopause has been estimated to be just under 4 years in some populations [10]. As a woman moves into the perimenopause, FSH can fluctuate between pre- and postmenopausal values and menopausal symptoms such as erratic menstrual cycles, and hot flashes will be experienced [11]. The variability of FSH levels during this time has called into question the reliability of using the FSH measure to diagnose or predict menopause [12]. However, in injectable users, it is the only means of assessing the menopause and, based on our findings, it should still be considered an option.

Further work will be done in a more in-depth study on this issue, which will involve taking multiple samples in a smaller subset of women who have agreed to participate in having repeat samples taken at short intervals within the dosing interval.

4.1. Limitations of the study

This study had a number of limitations. Subjects were part of another study which was not set up for taking multiple blood samples, which would be required to obtain an in-depth picture of gonadotrophin suppression. The researchers aimed to get subjects to come once during the peak gonadotrophin suppression period and once towards the end of a 2- or 3-month injection cycle. This was not always possible, as many women were only contactable by letter and it was therefore difficult to remind them to come at the exact time scheduled. The laboratory had not developed its own reference ranges for the FSH test.

Acknowledgments

This study was supported by a grant from the World Health Organisation and The Department for International Development UK (DFID). We wish to acknowledge the Chemical Pathology Department at the Nelson R. Mandela Medical School of the University of Natal for performing the assays for the study, Mrs. Fikile Mbatha for coordination of the study. We would also like to thank Dr. Olav Meirik for his support in developing the protocol.

References

- [1] World Health Organisation Special Programme of Research, Development and Research Training in Human Reproduction. Task Force on Long-acting Systemic Agents for the Regulation of Fertility. Multinational comparative clinical evaluation of two long-acting injectable contraceptive steroids: Norethisterone enanthate given in two dosage regimens and depot medroxyprogesterone acetate: Final report. *Contraception* 1983;23:1–20.
- [2] Guilleband J. *Contraception: your questions answered*. Edinburgh: Churchill Livingstone, 1993, p. 280.
- [3] Mishell DR, Khanna KM, Thorneycroft IH, Nakamura RM. Estrogenic activity in women receiving an injectable progestogen for contraception. *Am J Obstet Gynaecol* 1972;113:3726.
- [4] Franchimont P, Cession G, Ayalon D, Musters A, Legros JJ. Suppressive action of norethisterone enanthate and depo medroxyprogesterone acetate on gonadotropin levels. *Obstet Gynecol* 1970;36:93–100.
- [5] Fotherby K, Howard G, Srimanker K, Elder M, Bye PG. Effect of norethisterone oenanthate on serum gonadotrophin levels. *Contraception* 1977;16:591–604.
- [6] Perez-Palacios G, Chavez B, Escobar N, et al. Mechanism of action of contraceptive synthetic progestins. *J Steroid Biochem* 1981;15:125–30.
- [7] Department of Health South Africa and Macro International. *The South African Demographic and Health Survey, 1998. Preliminary report*. Department of Health Pretoria, 1999.
- [8] Mishell DR Jr. Pharmacokinetics of depot medroxyprogesterone acetate contraception. *J Reprod Med* 1996;41:381–90.
- [9] Fotherby K, Saxena BN, Shrimanker K, et al. A preliminary pharmacokinetic and pharmacodynamic evaluation of depot medroxyprogesterone acetate and norethisterone oenanthate. *Fertil Steril* 1980;34:131–8.
- [10] McKinley SM, Brambilla DJ, Posner JG. The normal menopause transition. *Maturitas* 1992;14:103–15.
- [11] Sherman BM, Korenman SG. Hormonal characteristics of the human menstrual cycle throughout reproductive life. *J Clin Invest* 1975;55:699–706.
- [12] Burger GH. Diagnostic role of follicle-stimulation hormone (FSH) measurements during the menopausal transition. An analysis of FSH, oestradiol and inhibin. *Eur J Endocrinol* 1994;130:38–42.

References

- Afghani, A., Abbot, A.V., Wiswell, R.A., et al. 2004. Bone mineral density in Hispanic women: role of aerobic capacity, fat-free mass, and adiposity. *Int J Sports Med*, vol.25, no.5, pp.384-90.
- Ahlborg, M.G., Johnell, O., Nilsson, B.E., et al. 2001. Bone loss in relation to menopause: a prospective study during 16 years. *Bone*, vol.28, no.3, pp.327-31.
- Aitken, J.M., Hart, D.M. & Lindsay, R. 1973. Oestrogen replacement therapy for prevention of osteoporosis after oophorectomy. *BMJ*, vol.3, no.5879, pp.515-8.
- Albertazzi, P., Bottazzi, M. & Steel, S.A. 2006. Bone mineral density and depot medroxyprogesterone acetate. *Contraception*, vol.73, no.6, pp.577-583.
- Alderman, B.W., Weiss, N.S., Daling, J.R., et al. 1986. Reproductive history and postmenopausal risk of hip and forearm fracture. *Am J Epidemiol*, vol.124, no.2, pp.262-7.
- American Health consultants. 1994. Depo-Provera often No.1 choice for teen-agers. *Contraceptive Technology Update*, Vol.19, pp.1-3.
- Bachrach, L.K., Cundy, T. & Ott, S.M. 2000. Depot medroxyprogesterone acetate in teens: a risk for bone health? *Pediatrics*, vol.106,no.5, pp.1137-8.
- Bahamondes, L., Juliato, C.T., Villarreal, M., et al. 2006a. Bone mineral density in users of two kinds of once-a-month combined injectable contraceptives. *Contraception*, vol.74, no.3, pp.259-263.
- Bahamondes, L., Espejo-Arce, X., Hildalgo, M.M., et al. 2006b. A cross-sectional study of the forearm bone density of users of long-term users of levonorgestrel- releasing intrauterine system. *Hum Reprod*, vol.21, no.5, pp.1316-9.

- Bahamondes, L., Monteiro-dantas, C., Espejo-Arce, X., et al. 2006c. A prospective study of the forearm bone density of users of etonorgestrel- and levonorgestrel-releasing contraceptive implants. *Hum Reprod*, vol.21, no.2, pp.466-70.
- Bahamondes, L., Perotti, M., Castro, S., et al. 1999. Forearm bone density in users of Depo-Provera as a contraceptive method. *Fertil Steril*, vol.71,no.5, pp.849-52.
- Barad, D., Koperberg, C., Wactawski-Wende, J., et al. 2005. Prior oral contraception and postmenopausal fracture: a Woman's Health Initiative observational cohort study. *Fertil Steril*, vol.84,no.2, pp.374-83.
- Baxter-Jones, A.D.G., Kontulainen, S.M., Faulkner, R.A., et al. 2008. A longitudinal study of the relationship of physical activity to bone mineral accrual from Adolescence to young adulthood. *Bone*, vol.43,no.6, pp.1101-07.
- Baxter-Jones, A.D.G., Burrows, M., Bachrach, L.K., et al. 2010. International pediatric reference standards for bone mineral content. *Bone*, vol.46, no.1, pp. 208-216.
- Beerthuizen, R., van Beek, A., Massai, R., et al. 2000. Bone mineral density during long term use of the progestagen contraceptive implant Implanon compared to non-hormonal method of contraception. *Hum Reprod*, vol.15, no.1, pp.118-122.
- Belsey, E.M. 1988. Vaginal bleeding patterns among women using one natural and eight hormonal methods of contraception. *Contraception*, vol.38, no.2, pp.181-206.
- Berenson, A.B., Breitkopf, C.R., Grady, J.J., et al. 2004. Effects of hormonal contraception on bone mineral density after 24 months of use. *Obstet Gynecol*, vol.103, no.5 pt. 1, pp.899-906.
- Berenson, A.B., Radecki, C.M., Grady, J.J, et al. 2001. 'A prospective, controlled study of the effects of hormonal contraception on bone mineral density. *Obstet Gynecol*, vol.98, no.4, pp.576-82.

- Berenson, A.B., Rahman, M., Breitkopf, C.R., et al. 2008. Effects of depot medroxyprogesterone acetate and 20-microgram oral contraceptives on bone mineral density. *Obstet Gynecol*, vol.112, no.4, pp.788-99.
- Berning, B., van, K.C., Schutte, H.E., et al. 1993. Determinants of lumbar bone mineral density in normal weight, non-smoking women soon after menopause. A study using clinical data and quantitative computed tomography. *Bone Miner*, vol.21,no.2, pp.129-39.
- Bigrigg, A., Evans, M., Gbolade, B., et al. 1999. Depo Provera, Position paper on clinical use, effectiveness and side effects. *Br J Fam Plann*, vol.25, no.2, pp.69-76.
- Black box warning added concerning long-term use of Depo-Provera contraceptive injection. FDA Talk Paper T04-50 November 17, 2004. <http://www.fda.gov/bbs/topics/ANSWERS/2004/ANS0135.HTML> accessed October 2, 2006.
- Burger, H.G. 1994. Diagnostic role of follicle-stimulation hormone (FSH) measurements during the menopausal transition- an analysis of FSH, oestrodial and inhibin. *Eur J Endocrinol*, vol.130,no.1, pp.38-42.
- Busen, N.H., Britt, R.B. & Rianon, N. 2003. Bone mineral density in a cohort of adolescent women using depot medroxyprogesterone acetate for one to two years. *J Adolesc Health*, vol.32, no.4, pp.257-9.
- Caird, L.E., Reid-Thomas, V., Hannan, W.J., et al. 1994. Oral progestogen-only contraception may protect against loss of bone mass in breast-feeding women. *Clin Endocrinol (Oxf)*, vol.41, no.6, pp.739-45.
- Cann, C., Genant, H.K., Ettinger, B., et al. 1980. Spinal mineral loss in Oophorectomized women. *JAMA*, vol.244,no.18, pp.2056-9.

- Castelo-Branco, C., Vicente, J.J., Pons, F., et al. 2001. Bone mineral density in young, hypothalamic oligoamenorrheic women treated with oral contraceptives. *J Reprod Med*, vol.46, no.10, pp.875-9.
- Chan, G.M., McMurry, M., Westover, K., et al. 1987. Effects of increased dietary calcium and bone mineral status of lactating adolescent and adult women. *Am J Clin Nutr*, vol.46, no.2, pp.319-23.
- Chotnopparatpattara, P. & Taneepanichskul, S. 2000. Use of depot medroxyprogesterone acetate in Thai adolescents. *Contraception*, vol.62, no.3, pp.137-140.
- Christiansen, C., Riis, B.J., Nilash, R.P., et al. 1985. Uncoupling of bone formation and resorption by combined oestrogen and progestagen therapy in postmenopausal osteoporosis. *Lancet*, vol.2, no.8459, pp.800-1.
- Clark, M.K., Sowers, M.R., Nichols, S., et al. 2004. Bone mineral density changes over two years in first-time users of depot medroxyprogesterone acetate. *Fertil Steril*, vol.82, no.6, pp.1580-6.
- Clark, M.K., Sowers, M.R., Levy, B., et al. 2006. Bone mineral density loss and recovery during 48 months in first-time users of depot medroxyprogesterone acetate. *Fertil Steril*, vol.86, no.5, pp. 1466-74.
- Cobb, K.L., Kelsey, J.L, Sidney, S., et al. 2002. Oral contraceptives and bone mineral density in white and black women in CARDIA. *Osteoporos Int*, vol.13, no.11, pp.893-900.
- Collins, C., Thomas, K., Harding, A., et al. 1988. The effect of oral contraceptives on lumbar bone density in premenopausal women. *J La State Med Soc*, vol.140, no.9, pp.35-9.
- Cooper, C., Hannford, P., Croft, P., et al. 1993. Oral contraceptive pill use and fractures in women: a prospective study. *Bone*, vol.14, no.1, pp.41-5.

- Cromer, B.A., Bonny, A.E., Stager, M., et al. 2008. Bone mineral density in adolescent females using injectable or oral contraceptives: A 24-month prospective study. *Fertil Steril*, vol. 90,no.6,pp.2060-7.
- Cromer, B.A., Lazebnik, R., Rome, E., et al. 2005. Double-blinded randomized controlled trial of oestrogen supplementation in adolescent girls who receive depot medroxyprogesterone acetate for contraception. *Am J Obstet Gynecol*, vol.192, no.1, pp.42-7.
- Cromer, B.A., Blair, J., Mahan, J.D., et al. 1996. A prospective comparison of bone density in adolescent girls receiving depot medroxyprogesterone acetate (Depo-Provera), levonorgesterel (Norplant), or oral contraceptives. *J Pediatr*, vol.129, no.5, pp. 671-6.
- Cromer, B.A. & Harel, Z. 2000. Adolescents: at increased risk of osteoporosis? *Clin Pediatr (Phila)*, vol.39,no.10, pp.565-74.
- Cromer, B.A., Stager, M., Bonny, A., et al. 2004. Depot medroxyprogesterone acetate, oral contraceptives and bone mineral density in a cohort of adolescent girls. *J Adolesc Health*, Dec. vol.35, no.6, pp.434- 41.
- Cromer, B.A. 2003. Bone mineral density in adolescent and young women on injectable or oral contraception. *Curr Opin Obstet Gynecol*, Vol.15, no.5, pp.353-7.
- Cundy, T., Ames, R., Horne, A., et al. 2003. A randomized controlled trial of oestrogen replacement therapy in long-term users of depot medroxyprogesterone acetate. *JCEM*, vol.88, no.1, pp.78-81.
- Cundy, T., Cornish, J., Roberts, H., et al. 2002. Menopausal bone loss in long-term users of depot medroxyprogesterone acetate contraception. *Am J Obstet Gynecol*, vol.186,no.5, pp. 978-83.

- Cundy, T., Cornish, J., Roberts, H., et al. 1998. Spinal bone density in women using depot medroxyprogesterone contraception. *Obstet Gynecol*, vol.92, no.4 pt. 1, pp.569-73.
- Cundy, T., Farquhar, C.M., Cornish, J., et al. 1996. Short-term effects of high dose oral medroxyprogesterone acetate on bone density in premenopausal women. *JCEM*, vol.81, no.3, pp.1014-7.
- Cundy, T., Cornish, J., Evans, M.C., et al. 1994. Recovery of bone density in women who stop using medroxyprogesterone acetate. *BMJ*, vol.308, no.6923, pp. 247-8.
- Cundy, T., Evans, M., Roberts, H., et al. 1991. Bone density in women receiving depot medroxyprogesterone acetate for contraception. *BMJ*, vol.303, no.6793, pp.13-16.
- Curtis, M.K. & Martins, S.L. 2006. Progestogen-only contraception and bone mineral density: a systematic review. *Contraception*, vol.73, no.5, pp.470-487.
- Dalsky, G.P. 1987. Exercise: Its effect on bone mineral content. *Clin Obstet Gynecol*, vol.30, no. 4, pp. 820-32.
- Davis, A.J. 1996. Use of depot medroxyprogesterone acetate contraception in adolescents. *J Reprod Med*, vol.41, no.5 (Suppl), pp. 407-413.
- De Cherney, A. 1996. Bone-spring properties of oral contraceptives. *Am J obstet Gynecol*, vol.174, no. 1 pt. 1, pp. 15-20.
- De Cherney, A. 1993, Physiologic and pharmacologic effects of oestrogen and progestins on bone. *J Reprod Med*, Dec; vol.38, no.12, Suppl, pp.1007-14.
- Dempster, D.W. 1995. Bone remodeling. In: Riggs BL, Melton III LJ eds Osteoporosis. Etiology, Diagnosis, and management. *Lippincott-Raven, Philadelphia*, pp.67-91.
- Department of Health, Medical Research Council, OrcMacro. 2007. *South Africa Demographic and Health Survey 2003*. Pretoria: Department of Health.

- Department of Health, Medical Research Council, OrcMacro. 2003. *South Africa Demographic and Health Survey 1998*. Pretoria: Department of Health.
- Di, X., Li, Y., Zhang, C., et al. 1999. Effects of levonorgestrel-releasing subdermal contraceptive implants on bone density and bone metabolism. *Contraception*, vol.60, no.3, pp.161-166.
- Diaz, S., Reyes, M.V., Zepeda, A., et al. 1999. Norplant implants and progesterone vaginal rings do not affect maternal bone turnover and density during lactation and after weaning. *Hum Reprod*, vol.14,no.10, pp.2499-2505.
- Duppe, H., Gradsell, P., Nilsson, B., et al. 1997. A single bone density measurement can predict fractures over 25 years. *Calcif Tissue Int*, vol.60 no.2, pp.171-4.
- Duursma, S.A., Raymakers, J.A., Boereboom, F.T.J., et al.1991. Estrogen and bone metabolism. *Obstet Gynecol Survey*, vol.47, pp.38-44.
- Eldred, J.M., Haynes, P.J. & Thomas, E.J. 1992. A randomized double blind placebo controlled trial of the effects on bone metabolism of the combination of nafarelin acetate and norethisterone. *Clin Endocrinol (Oxf)*, vol.37 no.4, pp. 354-9.
- El-Mahgoub, S. & Karim, M.1972. The long-term use of injectable norethisterone enanthate as a contraceptive. *Contraception*, vol.5, no. 1, pp.21-9.
- Elgan, C., Dykes, A.K. & Samsioe, G. 2004. Bone mineral density changes in young women: a two-year study. *Gynecol Endocrinol*, vol.19, no. 4, pp.169-77.
- Elgan, C., Samsioe, G. & Dykes, A.K. 2003. Influence of smoking and oral contraceptives on bone mineral density changes in young women: a 2-year study. *Contraception*, vol.67, no. 6, pp.439-47
- Endrikat, J., Mih, E., Düsterberg, B., et al. 2004. A 3 year double-blind, randomized, controlled study on the influence of two oral contraceptives containing either 20µg or

- 30µg ethinylestradiol in combination with levonorgestrel on bone mineral density. *Contraception*, vol.69, no. 3, pp.179-87.
- Enzelberger, H., Metka, M. & Heytmanek G. 1988. Influence of oral contraceptive use on bone density in climacteric women. *Maturitas*, vol.9, no. 4, pp.375-8.
- Ettinger, B., Genart, H.K., Cann, C.E. 1987. Post-menopausal bone loss is prevented by treatment with low dose estrogen with calcium. *Ann Intern Med*, vol.106, no. 1, pp.40-5.
- Faculty of Family Planning and Reproductive Health Care of The Royal College of Obstetricians and Gynaecologists. 2004. *Statement on MHRA guidance on Depo-Provera for prevention of postmenopausal bone loss*. FF PRHC 2004. http://www.ffprhc.org.uk/admin/uploads/Depo_Provera_alert_statement.pdf accessed October 2, 2006.
- Forsmo, S., Schei, B., Langhammer, A., et al. 2001. How do reproductive and lifestyle factors influence bone density in distal and ultradistal radius of early postmenopausal women? The Nord-Trondelag Health Survey, Norway. *Osteoporos Int*, vol.12, no. 3, pp.222-9.
- Fotherby, K., Howard, G., Srimanker, K., et al. 1977. Effect of norethisterone oenanthate on serum gonadotrophin levels. *Contraception*, vol.16, no. 6, pp.591-604.
- Fotherby, K., Saxena, B.N., Shrimanker, K., et al. 1980. A preliminary pharmacokinetic and pharmacodynamic evaluation of depot medroxyprogesterone acetate and norethisterone oenanthate. *Fertil Steril*, vol.34, no.2, pp.131-9.
- Fortney, J.A., Feldblum, P.J., Talmage R.V., et al. 1994. Bone mineral density and history of oral contraceptive use. *J Reprod Med*, vol.39, no.2, pp.105-9.
- Franchimont, P., Cession, G., Ayalon, D., et al. 1970. Suppressive action of norethisterone enanthate and depo medroxyprogesterone acetate on gonadotropin levels. *Obstet Gynecol*, vol.36, no. 1, pp.93-100.

- Fraser, D., Tiitinen, A., Affandi, B., et al. 1998. Norplant® consensus statement and background review. *Contraception*, vol.57, no. 1, pp.1-9.
- Fraser, I.S. & Weisberg, E. 1982. Fertility following discontinuation of different methods of fertility control. *Contraception*, vol.26, no. 4, pp.389-415.
- Gallager, J.C., Kable, W.T. & Goldgar, D. 1991. Effect of progestin therapy on cortical and trabecular bone: Comparison with oestrogen. *Am J Med*, vol.90, no. 2, pp. 171-8.
- Gambacciani, M., Cappagli, B., Ciaponi, M., et al. 1999 (Spring). Hormone replacement therapy in perimenopause: the effect of low dose oral contraceptive preparation on bone quantitative ultrasound characteristics. *Menopause*, vol.6, no. 1, pp.43-8.
- Gambacciani, M., Ciaponi, M., Cappagli, B., et al. 2000. Longitudinal evaluation of perimenopausal femoral bone loss: effects of a low-dose oral contraceptive preparation on bone mineral density and metabolism. *Osteoporos Int*, vol.11, no. 6, pp.544-8.
- Gambacciani, M., Spinetti, A., Cappagli, B., et al. 1994a. Hormone replacement therapy in perimenopausal women with low dose oral contraceptive preparation: effects on bone mineral density and metabolism. *Maturitas*, vol.19, no.2, pp. 125-31.
- Gambacciani, M., Spinetti, A., Taponeco, F., et al. 1994b. Longitudinal evaluation of perimenopausal vertebral bone loss: effects of a low-dose oral contraceptive preparation on bone mineral density and metabolism. *Obstet Gynecol*, vol.83, no.3, pp.392-6.
- Gargano, V., Massaro, M., Morra, I., et al. 2008. Effects of two low-dose combined oral contraceptives containing drospirenone on bone turnover and bone mineral density in young fertile women: a prospective controlled randomized study. *Contraception*, vol. 78, no. 1, pp.10-15.
- Garnero, P., Sornay-Rendu, E. & Delmas, P.D. 1995. Decreased bone turnover in oral contraceptive users. *Bone*, vol.16, no.5, pp.499-503.

- Gbolade, B., Ellis, S., Murby, B., et al. 1998. Bone density in long-term users of depot medroxyprogesterone acetate. *Br J Obstet Gynecol*, vol.105, no. 7, pp.790-794.
- Genant, H.K., Ettinger, B., Harris, S.T., et al. 1988. Quantitative computed tomography in assessment of osteoporosis. In: Riggs BL, Melton III LJ eds Osteoporosis. Etiology, Diagnosis, and management. *Raven Press, New York*, pp.221-249.
- Goldsmith, N.F. & Johnston J.O. 1975. Bone Mineral Effects of Oral Contraceptives, Pregnancy and Lactation. *J Bone Joint Surg*, vol.57-A, no.5, pp.657-68.
- Goldsmith, N.F. & Baumberger, J.P. 1967. Mineral changes after Norethynodrel. *Lancet*, vol.2, no. 7515, pp. 567-568.
- Goldsmith, N.F. & Goldsmith, J.R. 1966. Epidemiological Aspects of Magnesium and Calcium Metabolism. Implications of Altered Magnesium Metabolism in women Taking Drugs for the Suppression of Ovulation. *Arch Environ Health*, vol.12, no. 5, pp.607-619.
- Grainge, M.J., Coupland, C.A.C., Cliffe, S.J., et al. 2001. Reproductive, menstrual and menopausal factors: which are associated with bone mineral density in early postmenopausal women? *Osteoporos Int*, vol.12, no. 9, pp.777-87.
- Guilbert, E.R., Brown J.P., Kaunitz, et al. 2009. The use of depot-medroxyprogesterone acetate in contraception and its potential impact on skeletal health. *Contraception*, vol.79, no. 3, pp.167-77.
- Guillebaud, J. 2001. Contraception: your questions answered. *Edinburg : Churchill Livingstone: 1993*. pp.280.
- Haenggi, W., Casey, J.P., Birkhauser, M., et al. 1994. Bone mineral density in young women with long-standing amenorrhea: limited effect of hormone replacement therapy with ethinylestradiol and desogestrel. *Osteoporos Int*, vol.4, no. 2, pp.99-103.

- Hall, M.L., Heavens, J., Cullum, I.D., et al. 1990. The range of bone density in normal British women. *Br J Radiol*, vol.63, no. 748, pp. 266-69.
- Hammond, C. B. & Maxson, W. S. 1982. Current status of oestrogen therapy for the Menopause. *Fertil Steril*, vol.37, no. 1, pp.5-25.
- Hansen, M., Overgaard, K., Riis, B., et al. 1991. Potential risk factors for development of postmenopausal osteoporosis-examined over a 12 year period. *Osteoporos Int*, vol.1, no. 2, pp.95-102.
- Hansen, M.A. 1994. Assessment of age and risk factors on bone density and bone turnover in healthy premenopausal women. *Osteoporos Int*, vol.4, no. 3, pp.123-8.
- Harris, R., Helfand, M., Woolf, S.H., et al. 2001. Current methods of the US preventative Services Task Force: a review of the process. *Am J Prev Med*, vol.20, no.3 (Suppl), pp.21-35.
- Hartard, M., Bottermann, P., Bartenstein, P., et al. 1997. Effects on bone mineral density of low-dosed oral contraceptives compared to and combined with physical activity. *Contraception*, vol.55, no. 2, pp.87–90.
- Hartard, M., Kleinmond, C., Lupp P., et al. 2006. Comparison of the skeletal effects of the progestogens desogestrel and levonorgestrel in oral contraceptive preparations in young women: controlled, open, partly randomized investigation over 13 cycles. *Contraception*, vol.74, no.5, pp.367-75.
- Hartard, M., Kleinmond, C., Wiseman, M., et al. 2007. Detrimental effect of oral contraceptives on parameters of bone mass and geometry in a cohort of 248 young women. *Bone*, vol.40, no. 2, pp.444-50.

- Hawker, G.A., Forsmo, S., Cadarette, S.M., et al. 2002. Correlates of forearm bone mineral density in young Norwegian women: the Nord-Trondelag health study. *Am J Epidemiol*, vol.156, no. 5, pp.418-27.
- Hayes, W.C., Piazza, S.J., Zysset, P.K. 1991. Biomechanics of fracture risk prediction of the hip and spine by quantitative computed tomography. *Radiol Clin North Am*, vol.29, no. 1, pp.1-18.
- Hayslip, C.C., Klein, T.A., Wray, H.L., et al. 1989. The effects of lactation on bone mineral content in healthy post partum women. *Obstet Gynecol*, vol.73, no. 4, pp.588-92.
- Horowitz, M., Wishart, J.M., Need, A.G., et al. 1993. Effects of norethisterone on bone related biochemical variables and forearm bone mineral density in postmenopausal osteoporosis. *Clin Endocrinol (Oxf)*, vol.39, no.6, pp.649-655.
- Hreshchyshyn, M.M, Hopkins, A., Zylstra, S., et al. 1988. Associations of parity, breast-feeding, and birth control pills with lumbar spine and femoral neck bone densities. *Am J Obstet Gynecol*, vol.159, no.2, pp.318-22.
- Hui, S. L., Wiske, P.S., Norton, J.A., et al. 1982. A prospective study of change in bone mass with age in postmenopausal women. *J Chronic Dis*, vol.35, no. 9, pp.715-25.
- Intaraprasert, S., Taneepanichskul, S., Theppisai, U., et al. 1997. Bone Density in women receiving Norplant implants for contraception. *J Med Assoc Thai*, vol.80, no.11, pp.738-41.
- Johansson, C. & Mellstrom, D. 1996. An earlier fracture as a risk factor for new fracture and its association with smoking and menopausal age in women. *Maturitas*, vol.24, no. 1-2, pp.97-106.
- Johnell, O, & Nilsson, B.E. 1984. Life-style and bone mineral mass in perimenopausal women. *Calcif Tissue Int*, vol.36, no. 4, pp.354-6.

- Johnson, C.C., Burkman R.T., Gold, M.A., et al. 2008. Longitudinal study of depot medroxyprogesterone acetate Depo-Provera ® effects on bone health in adolescents: study design, population characteristics and baseline bone mineral density. *Contraception*, Apr, vol.77, no. 4, pp.239-48.
- Johnston, C.C., Miller, J.Z. & Slemenda, C.W. 1992. Calcium supplementation and increases in bone mineral density in children. *NEJM*, vol.327, no. 2, pp.82-7.
- Kanders, B., Lindsay, R., Dempster, D., et al. 1984. Determinants of bone mass in young healthy women. In: Christiansen, C., Arnaud, C.D., Nordin, B.E.C., Parfitt, A.M., Peck, W.A. and Riggs, B.L., eds osteoporosis: proceedings of the Copenhagen international symposium on osteoporosis. *Copenhagen*, pp.337-9.
- Kass-Wolff, J.H., 2001. Bone loss in adolescents using Depo-Provera. *J Soc Pediatr Nurs*, vol.6, no.1, pp. 21-31.
- Kaunitz, A.M., Arias, R., McClung, M., et al. 2008, Bone density recovery after depot medroxyprogesterone acetate injectable contraception use. *Contraception*, vol.77, no.2, pp.67-76.
- Kaunitz, A.M., Miller, P.D., Montgomery, R.V., et al. 2006. Bone mineral density in women aged 25-35 years receiving depot medroxyprogesterone acetate: recovery following discontinuation. *Contraception*, vol.74, no. 2, pp.90-99
- Kaunitz, A.M., 1994. Long-acting injectable contraception with depot medroxyprogesterone acetate. *Am J Obstet Gynecol*, vol.170, no.5 pt. 2, pp.1543-9.
- Kleerekoper, M., Brienza, R.S., Schultz, L.R., et al. 1991. Oral contraceptive use may protect against low bone mass. *Arch Intern Med*, vol.151, no. 10, pp.1971-6.

- Kritz-Silverstein, D. & Barrett-Connor, E. 1993. Bone mineral density in postmenopausal women as determined by prior oral contraceptive use. *Am J Public Health*, vol.83,no.1, pp.100-2.
- Krolner, B., Toft, B., Nielsen, S.P., et al. 1983. Physical exercise as prophylaxis against involutional vertebral bone loss: a controlled trial. *Clin Sci (Lond)*, vol.64, no. 5, pp.541-546.
- La Vecchia, C., Tavani, A., & Gallus, S. 1999, Oral contraceptives and risk of hip fractures. *Lancet*, vol.354, no. 9175, pp.325-6.
- Laitinen, K., Valimaki, M. & Keto P. 1991, Bone mineral density measured by dual-energy X-ray absorptiometry in healthy Finnish women. *Calcif Tissue Int*, vol.48, no. 4, pp.224 - 31.
- Lamke, B., Brundin, J., Moberg, P., 1977, Changes of bone mineral content during pregnancy and lactation. *Acta Obstet Gynecol Scand*, vol.56, no. 3, pp.217-9.
- Lappe, J.M., Kimmel, D.B. & Stegman, M.R. 1997, Depo-medroxyprogesterone acetate contraception exposure and stress fractures in female military recruits. *JBMR*, vol.12, Suppl., pp.S173.
- Lara-tore, E., Edwards, C.P., Perlman, S.P., Paige, et al. 2004, Bone mineral density in adolescent females using depot medroxyprogesterone acetate. *J Pediatr Adolesc Gynecol*, vol.17, no. 1, p.17-21.
- Lewiecki, E., Gordon, S., Baim, M., et al. 2007, International Society for Clinical Densitometry 2007 Adult and Pediatric Official Positions. *Bone*, vol. 43,no. 6, pp.115-21.
- Lindsay, R., Hart, D.M. & Clark, D.M. 1984, The minimum effective dose of oestrogen for prevention of postmenopausal bone loss. *Obstet Gynecol*, vol.63, no. 6, pp.759-63

- Lindsay, R., Hart, D.M., Forrest, C., et al. 1980, Prevention of spinal osteoporosis in oophorectomized women. *Lancet*, vol.2, no. 8205, pp.1151-4.
- Lindsay, R., Tohme, J. & Kanders, B. 1986, The effect of oral contraceptive use on vertebral bone mass in pre-and post-menopausal women. *Contraception*, vol. 34, no. 4, 333-40.
- Lindsay, R. 1987, The menopause: sex steroids and osteoporosis. *Clin Obstet Gynecol*, vol.30, no. 4, pp.847-59.
- Lloyd, T., Buchanan, J.R., Ursino, G.R., et al. 1989, Long term oral contraceptive use does not affect trabecular bone density. *Am J Obstet Gynecol*, vol.160, no.2, pp.402-4.
- Lloyd, T., Taylor, D.S., Lin, H.M., et al. 2000. Oral contraceptive use by teenage women does not affect peak bone mass: a longitudinal study. *Fertil Steril*, vol.74, no. 4, pp.734- 8.
- Lloyd, T., Petit, M.A., Lin, H.M., et al. 2004. Lifestyle factors and the development of bone mass and bone strength in young women. *J Pediatr*, vol.144, no. 6, pp.776- 82.
- Lopez, L.M., Grimes, D.A., Schulz, K.F., et al. 2006. Steroidal contraceptives : effect on bone fractures in women. *Cochrane database of Systematic Reviews*, Issue 4. Art. No.:CD006033.DOI:10.1002/14651858.CD006033.pub2.
- MacDougall, J., Davies, M.C., Overton, C.E., et al. 1999. Bone density in a population of long term oral contraception pill users does not differ from that in menstruating women. *Br J Fam Plann*, vol.25, no. 3, pp.96-100.
- Mais, V., Fruzzetti, F., Ajossa, S., et al. 1993. Bone metabolism in young women taking a monophasic pill containing 20 µg ethinylestradiol: a prospective study. *Contraception*, vol.48, no. 5, pp.445-52.
- Mallmin, H., Ljunghall, S., Persson, I., et al.1994. Risk factors for fractures of the distal forearm: a population-based case-control study. *Osteoporos Int*, vol.4, no. 6, pp. 298-304.

- Margulies, R. & Miller, L. 2001. Increased depot medroxyprogesterone acetate use increases family planning program pharmaceutical supply costs. *Contraception*, vol.63, no. 3, pp.147-149.
- Martins, S.L., Curtis, K.M. & Glasier, A.F. 2006. Combined hormonal contraception and bone health: a systematic review. *Contraception*, vol.73, no. 5, pp.445-469.
- Masaryk, P., Lunt, M., Benevolenskaya, L., et al.1998 Effects of menstrual history and use of medications on bone mineral density: the EVOS study. *Calcif Tissue Int*, vol.63, no. 4, pp.271-6.
- Mazess, R.B. & Barden, H.S. 1991. Bone density in premenopausal women: effects of age, dietary intake, physical activity, smoking and birth control pills. *Am J Clin Nutr*, vol.53, no. 1, pp.132-42.
- Mazess, R.B. 1982. On aging bone loss. *Clin Orthop*, vol.165, pp.239-52.
- McGough, P., Bigrigg, A. 2007. Effect of medroxyprogesterone acetate on bone density in a Scottish industrial city. *Eur J Contracept Reprod Health Care*, vol.12, no.3, pp.253-259.
- Mckinley, S.M., Brambilla, D.J. & Posner J.G. 1992. The normal menopause transition. *Maturitas*, vol.14, no. 2, pp.103-15.
- Mehta, S. 1993. Bone loss, contraception and lactation. *Acta Obstet Gynecol Scand*, vol.72, no. 3, pp.148-56.
- Melton III, L.J., Bryant, S.C., Wahner, H.W., et al. 1993. Influence of breastfeeding and other reproductive factors on bone mass later in life. *Osteoporos Int*, vol.3, no. 2, pp. 76-83.

- Merki-Feld, G.S., Neff, M. & Keller P.J. 2003. A 2-year prospective study on the effects of depot medroxyprogesterone acetate on bone mass-response to oestrogen and calcium therapy in individual users. *Contraception*, vol.67, no. 2, pp.79-86.
- Michaelsson, K., Baron, J.A., Farahmand, B.Y., et al. 1999. Oral contraceptive use and risk of hip fracture: a case-control study. *Lancet*, vol.353, no. 9163, pp.1481-4.
- Michaësson, K., Baron, J.A., Farahmand, B.Y., et al. 2001. Influence of parity and lactation on hip fracture risk. *Am J Epidemiol*, vol.153, no.312, no. 12, pp.1166-1172.
- Mishell, D.R. 1996. Pharmacokinetics of depot medroxyprogesterone acetate contraception. *J Reprod Med*, vol.41, no.5 (Suppl.), pp.381-90.
- Mishell, D.R., Kharma, K.M., Thorneycroft, I.H., et al. 1972. Estrogenic activity in women receiving an injectable progesterone for contraception. *Am J Obstet Gynecol*, vol.113, no.3, pp.372-376.
- Monteiro-Dantas, C., Espejo-Arce, X., Lui-Filho, J.F., et al. 2007. A three-year longitudinal evaluation of the forearm bone density of users of etonogestrel-and levonorgestrel-releasing contraceptive implants. *Reprod Health*, vol.12, no.4, pp.11.
- Morrison, N.A., Cheng, Q.I., J.C., Tokiyata, A., et al. 1994. Prediction of bone density from vitamin D receptor alleles. *Nature*, vol.367, no. 6460, pp.284-287.
- Munk-Jensen, N., Pors-Nielsen, S., Obel, E.B., et al. 1988. Reversal of Post-menopausal vertebral bone loss by estrogen and progestogen: a double blind placebo controlled study. *BMJ (Clin Res Ed)*, vol.296, no. 6630, pp.1150-2.
- Murphy, S., Khaw, K.T.& Compston, J.E. 1993. Lack of relationship between hip and spine bone mineral density and oral contraceptive use. *Eur J Clin Invest*, vol.23, no. 2, pp.108-11.

- Naessen, T., Olsson, S.E. & Gudmundson, J. 1995. Differential effects on bone density of progestogen-only methods for contraception in premenopausal women. *Contraception*, vol.52, no. 1, pp.35-9.
- Nappi, C., Di Spiezie, S.A., Acunzo, G., et al. 2003. Effects of low-dose and ultra- low-dose combined oral contraceptives use on bone turnover and bone mineral density in young fertile women: a prospective controlled randomized study. *Contraception*, vol.67, no. 5, pp.355-9.
- Nappi, C., Di Spiezie. S.A., Tommaselli, G.A., et al. 2005. Effects of an oral contraceptive containing drospirenone on bone turnover and bone mineral density. *Obstet Gynecol*, vol.105, no. 1, pp.53-60.
- Need, A.G. & Nordin, B.E. 1990. Which bone to measure? *Osteoporos Int*, vol.1, no.1,pp.3-6.
- Nelson, M., Mayer, A.B., Rutherford, O., et al. 1991, Calcium intake, physical activity and bone mass in pre-menopausal women. *J Hum Nutr Diet*, vol.4, pp.171-8.
- Nordin, B.E.C. 1987 The definition and diagnosis of osteoporosis. *Calcif Tissue Int*, vol.40,no. 2, pp.57-8.
- Lader, D., Hopkins, G., 2007/08. Contraception and Sexual Health: A report on research using the National Statistics Omnibus Survey produced on behalf of the NHS Information Centre for health and social care. Office for National Statistics, *Omnibus Survey Report* No.37, Newport,UK.
- O'Neill, T.W., Marsden, D., Adams, J.E., et al. 1996. Risk factors, falls, and fracture of the distal forearm in Manchester, UK. *JECH*, vol.50, no. 3, pp.288-92.

- Ortiz, A., Hiroi, F., Stanczyk, F.Z., et al. 1977. Serum medroxyprogesterone acetate MPA concentration and ovarian function following intramuscular injection of Depo-MPA. *JCEM*, vol.44, no.1, pp.32-8.
- Orr-Walker, B.J., Evans, M.C., Ames, R.W., et al. 1998. The effect of past use of the injectable contraceptive depot medroxyprogesterone acetate on bone mineral density in normal post-menopausal women. *Clin Endocrinol (Oxf)*, vol.49, no. 5, pp.615-618.
- Ott, S.M., Scholes, D., Lacroix, A.Z., et al. 2001. Effects of contraceptive use on bone biochemical markers in young women. *JCEM*, vol.86, no.1, pp.179- 85.
- Paoletti, A.M., Orru, M., Lello, S., et al. 2004. Short-term variations in bone remodeling markers of an oral contraception formulation containing 3mg of drosoirenone plus 30 microg of ethyiny l estradiol: observational study in young postadolescent women. *Contraception*, vol.70, no. 4, pp.293-8.
- Parfitt, A.M. 1988. Bone remodelling: relationship to the amount and structure of bone, and the pathogenesis and prevention of fractures. In: Riggs BL, Melton III LJ eds Osteoporosis. Etiology, Diagnosis, and management. *Raven Press, New York*, pp.221-249.
- Pasco, J.A., Kotowicz, M.A., Henry, M.J., et al. 2000. Oral contraceptives and bone mineral density: a population-based study. *Am J Obstet Gynecol*, vol.182, no.2, pp.265-9.
- Pavia, L.C., Pinto-Neto, A.M.& Faundes A. 1998. Bone density among long-term users of medroxyprogesterone acetate as a contraceptive. *Contraception*, vol.58, no. 6, pp. 351-5.
- Perez-Palacois, G., Chavez, B., Escobar, N., et al. 1981. Mechanism of action of contraceptives synthetic progestins. *J Steroid Biochem*, vol.15, pp.125-30.

- Perrotti, M., Bahamondes, L., Petta, C., et al. 2001. Forearm bone density in long-term users of oral combined contraceptives and depot medroxyprogesteron acetate. *Fertil Steril*, vol.76, no.3, pp.469-73.
- Petitti, D.B., Piaggio, G., Mehta, S., et al. 2000. Steroid Hormone Contraception and bone mineral density: A cross-Sectional study in an International Population. *Obstet Gynecol*, vol.95, no.5, pp.736-44.
- Picard, D., Ste-Marie, L.G., Coutu, D., et al. 1988. Premenopausal bone mineral content relates to height, weight and calcium intake during early adulthood. *Bone Miner*, vol.4, no. 3, pp.299-309.
- Pikkarainen, E., Lehtonen-Veromaa, M., Mottonen, T., et al. 2008. Estrogen- progestin contraceptive use during adolescence prevents bone mass acquisition: a 4-year follow-up study. *Contraception*, vol.78,no.3, pp.226-31.
- Polaneczky, M., Liblanc, M. 1998. Long-term depot medroxyprogesterone acetate Depo-Provera use in inner-city adolescents. *J Adolesc Health*, vol.23, no.2, pp.81-8
- Polatti, F., Perotti, F., Felippa, N., et al. 1995. Bone mass and long-term monophasic oral contraceptive treatment in young women. *Contraception*, vol.51, no. 4, pp.221-4.
- Prior, J.C., Kirkland, S.A., Joseph, L., et al. 2001. Oral contraceptive use and bone mineral density in premenopausal women: cross-sectional, population-based data from the Canadian Multicentre Osteoporosis Study. *CMAJ*, vol.165,no.8, pp.1023-9.
- Recker, R.R., Davies, K.M., Hinders, S.M., et al. 1992. Bone gain in young adult women. *JAMA*, vol.268, no.17, pp.2403-8.
- Reed, S.D., Scholes, D., LaCroix, A.Z., et al. 2003. Longitudinal changes in bone density in relation to oral contraceptive use. *Contraception*, vol.68, no. 3, pp.177-182.

- Reproductive Health Task Force, South African Ministry of Health, WHO. 1994. Assessment of reproductive health in South Africa focusing on family planning. Unpublished Report.
- Richelson, L.S., Wahner, H.W., Melton, L.J., et al. 1984. Relative contributions of aging and oestrogen deficiency to post-menopausal bone loss. *NEJM*, vol.311, no. 20, pp.1273-5.
- Riggs, B.L. & Melton III, L.J. 1986. Involutional osteoporosis. *NEJM*, vol.314, pp.1676-686.
- Riggs, L., Wahner, H.W., Melton III, L. J., et al. 1986. Rates of bone loss in the appendicular and axial skeletons of women. *J Clin Invest*, vol.77, no. 5, pp.1487-91.
- Riis, B.J., Christiansen, C., Johansen, J.S., et al. 1990. Is it possible to prevent bone loss in young women treated with lutenizing hormone-releasing hormone agonists? *JCEM*, vol.70, no.4, pp.920-4.
- Rodin, A., Chapman, M. & Fogelman, I. 1991. Bone density in users of combined oral Contraception. *Br J Fam Plann*, vol.16, no. 4, pp. 125-9.
- Rodin, A., Murby, B., Smith, M.A., et al. 1990. Perimenopausal bone loss in the lumbar spine and neck of femur: a study of 225 Caucasian women. *Bone*, vol.11,no.1, pp.1-5.
- Rome, E., Ziegler, J., Secic, M., et al. 2004. Bone biochemical markers in adolescent girls using either depot medroxyprogesterone acetate or an oral contraceptive. *J Pediatr Adolesc Gynecol*, Dec; vol.17, no. 6, pp.373-7.
- Rosenburg, L., Zhang, Y., Constant, D., et al. 2007. Bone status after cessation of use of injectable progestin contraceptives. *Contraception*, vol.76, no. 6, pp.425-31
- Sabatier, J.P., Guaydier-Souquieres, G., Laroche, D., et al. 1996. Bone mineral acquisition during adolescence and early childhood: a study in 574 healthy females 10-24 years of age. *Osteoporos Int*, vol.6, no. 2, pp.141-8.

- Sanches, L., Marchi, N.M., Castro, S., et al. 2008. Forearm bone mineral density in postmenopausal former users of depot medroxyprogesterone acetate. *Contraception*, vol.78, no. 5, pp.365-9.
- Schnitzer, C.M., MacPhail, A.P., Shires, R., et al. 1994. Osteoporosis in African hemosiderosis: role of alcohol and iron. *J Bone Miner Res*, vol.9, no. 12, pp.1865-1873.
- Scholes, D., Lacroix, A.Z., Ichikawa, L.E., et al. 2005. Change in bone mineral density among adolescent women using and discontinuing depot medroxyprogesterone acetate contraception. *Arch Pediatr Adolesc Med*, vol.159, no. 2, pp.139-144.
- Scholes, D., LaCroix, A.Z., Ichikawa L.E., et al. 2004. The association between depot medroxyprogesterone acetate contraception and bone mineral density in adolescent women. *Contraception*, vol.69, no. 2, pp.99-104.
- Scholes, D., LaCroix, A.Z., Ichikawa L.E., S.M., et al. 2002. Injectable hormone contraception and bone density: results from a prospective study. *Epidemiology*, vol.13, no. 5, pp.581-7.
- Scholes, D., Lacroix, A.Z, Ott, S.M., et al. 1999. Bone mineral density in women using depot medroxyprogesterone acetate for contraception. *Obstet Gynecol*, vol.93, no. 2, pp.233-238.
- Seeman, E., Szumukler, G.I., Formica, C., et al. 1992. Osteoporosis in anorexia nervosa: the influence of peak bone density, bone loss, oral contraceptive use, and exercise. *J Bone Miner Res*, vol.7, no. 12, pp.1467-74.
- Shaarawy, M., El-Mallah, S., Seoudi, S., et al. 2006. Effects of the long-term use of depot medroxyprogesterone acetate as hormonal contraceptive on bone mineral density and biochemical markers of bone remodeling. *Contraception*, vol.74, no. 4, pp.297-302.

- Shargil, A.A. 1985. Hormone replacement Therapy in perimenopausal women with a triphasic contraceptive compound: a three-year prospective study. *Int J fertile*, vol.30, no. 1:15, pp.18-28.
- Sherman, B.M. & Korenman, S.G. 1975. Hormonal characteristics of the human menstrual cycle throughout reproductive life. *J Clin Invest*, vol.55, no. 4, pp.699-706.
- Siddle, N., Sarrel. P. & Whitehead, M. 1987. The effect of hysterectomy on the age of ovarian failure: identification of a subgroup of women with premature loss of ovarian function, and literature review. *Fertil Steril*, vol.47, no. 1, pp.94-100.
- Silverstein, D.K., Connor, E.B. & Hollenbach, K.A. 1992. Pregnancy and lactation as determinants of BMD in postmenopausal women. *Am J of Epidemiol*, vol.136, no. 9, pp.1052-59.
- Simpson, G.R. & Dale, E. 1972. Serum levels of Phosphorous, magnesium, and calcium in women utilizing Combination Oral or long-acting injectable Progestational Contraceptives. *Fertil Steril*, vol.23, no. 5, pp.326-330.
- Slemenda, C.W., Miller, J.Z., Hui, S.L., et al. 1991. Role of physical activity in the development of skeletal mass in children. *JBMR*, vol.6, no. 11, pp.1227-33.
- Smit, J., Gray, A.L., McFadyen, M.L., et al. 2001 Counting the costs: comparing depot medroxyprogesterone acetate and norethisterone oenanthate utilisation patterns in South Africa. *BMC Health Services Research*, vol.1(1), no.4.
- Society for Adolescent Medicine. 2006. Depot Medroxyprogesterone Acetate and Bone Mineral density in Adolescents- The Black Box warning: A position paper of the Society for Adolescent Medicine. *J Adolesc Health*, vol.39, no. 2, pp.296-301.
- Sowers, M.R., Wallace, R.B. & Lemke, J.H. 1985. Correlates of forearm bone mass among women during maximal bone mineralization. *Prev Med*, vol.14, no. 5, pp.585-96.

- Sowers, M.R. & Galuska, D.A. 1993. Epidemiology of bone mass in premenopausal women. *Epidemiol Rev*, vol.15,no. 2, pp.374-98.
- Sowers, M.F., Corton, G., Shapiro, B., et al. 1993. Changes in bone density with lactation. *JAMA*, vol.269, no. 24, pp.3130-5.
- Stevenson, J.C., Less, B., Devenport, M., et al. 1989. Determinants of bone-density in normal women: risk factors for future osteoporosis. *BMJ*, vol.298, no. 6678, pp.924-8.
- Sultana, S., Choudhury, S. & Chaoudhur, S.A. 2002. Effect of combined oral contraceptives on bone mineral density in pre-and postmenopausal women. *Mymensingh Med J*, vol. 11,no. 1, pp.12-4.
- Szarewski, A., Gillebaud, J. & Christopher, E. 1991. DMPA and bone density. *BMJ*, 6806, pp.303-467.
- Szarewski, A. & Mansour, D. 1999. Spinal Bone density in Women Using Depot Medroxyprogesterone Acetate. *Obstet Gynecol*, vol.93, vol.4, pp.629-30.
- Taechakraichana, N., Limpaphayom, K., Ninlagarn, T., et al. 2000. A randomized trial of oral contraceptive and hormone replacement therapy on bone mineral density and coronary heart disease risk factors in postmenopausal women. *Obstet Gynecol*, vol.95, no. 1, pp.87-94.
- Tamaki, J., Iki, M., Sato, Y., et al. 2009. Smoking among premenopausal women is associated with increased risk of low bone status:the JPOS Study. *J Bone Miner Metab*, [Epub ahead of Print}.
- Taneepanichskul, S., Intaraprasert, S., Theppisai, U., et al. 1997a. Bone mineral density during long-term treatment with Norplant® implants and depot medroxyprogesterone acetate. A cross-sectional study of Thai women. *Contraception*, vol.56 no.3, pp.153-155.

- Taneepanichskul, S., Intaraprasert, S., Theppisai, U., et al. 1997b. Bone mineral density in long-term depot-medroxyprogesterone acetate acceptors. *Contraception*, vol.56, no. 1, pp.1-3.
- Tang, O.S., Tang, G., Yip, P., et al. 1999, Long-term depot-medroxyprogesterone acetate and bone mineral density. *Contraception*, vol.59, no. 1, pp.25-9.
- Tang, O.S., Tang, G., Yip, P.S.F., et al. 2000. Further evaluation on long-term depot-medroxyprogesterone acetate use and bone mineral density; a longitudinal cohort study. *Contraception*, vol.62, no. 4, pp.161-4.
- Tharnprisarn, W. & Taneepanichskul, S. 2002. Bone mineral density in adolescent and young Thai girls receiving oral contraceptives compared with depot medroxyprogesterone acetate: a cross-sectional study in young Thai women. *Contraception*, vol.66, no. 2, pp.101-3.
- Theintz, G., Buchs, B., Rizzoli, R., et al. 1992. Longitudinal monitoring of bone mass accumulation in healthy adolescents: Evidence for marked reduction after 16 years of age at the levels of the lumbar spine and femoral neck in female subjects. *JCEM*, vol.75, no. 4, pp.1060-5
- Tuppurainen, M., Kröger, H., Saarikoski, S., et al. 1994. The effect of previous oral contraceptive use on bone mineral density in perimenopausal women. *Osteoporos Int*, vol.4, no. 2, pp.93-98.
- Ulrich, C.M., Georgiou, C.C., Snow-Harter, C.M., et al. 1996. Bone mineral density in mother-daughter pairs: relations to lifetime exercise, lifetime milk consumption, and calcium supplements. *Am J Clin Nutr*, vol.63, no. 1, pp.72-9.

- Vanderjagt, D.J., Sagay, A.S., Imade, G.E., et al. 2005. Effect of Norplant contraceptive on the bones of Nigerian women as assessed by quantitative ultrasound and serum markers of bone turnover. *Contraception*, vol.72, no.3, pp.212-6.
- Vessey, M., Mant, J. & Painter, R. 1998. Oral contraception and other factors in relation to hospital referral for fracture. Findings in a large cohort study. *Contraception*, vol.57, no. 4, pp.231-5.
- Virutamasen, P., Wangsuphachart, S., Reinprayoon, D., et al. 1994. Trabecular bone in long-term depot-medroxyprogesterone acetate users. *Asia-Oceania J Obstet Gynaecol*, vol.20, no. 3, pp.269-74.
- Volpe, A., Amram, A., Cagnacci, A., et al. 1997. Biochemical aspects of hormonal contraception: effects on bone metabolism. *Eur J Contracept Reprod Health Care*, vol.2, no. 2, pp.123-6.
- Von Kesseru, E., Etchepareborda, J.J., Wikinski, R., et al. 2000. Premenopause contraception with monthly injectable Mesigyna with special Emphasis on serum lipid and bone density patterns. *Contraception*, vol.61, no. 5, pp.317-322.
- Walsh, J.S., Eastell, R. & Peel, N.F. 2008a. Depot medroxyprogesterone acetate use after peak bone mass is associated with increased bone turnover but no decrease in bone mineral density. *Fertil Steril*, Nov eupub.
- Walsh, J.S., Eastell, R. & Peel, N.F. 2008b. Effects of Depot medroxyprogesterone acetate on bone density and bone metabolism before and after peak bone mass: A Case-Control Study. *JCEM*, vol.93, no.4, pp.1317-23
- Wanichsetakul, P., Kamudhamas, A., Watanaruangkovit, P., et al. 2000. Bone mineral density at various anatomic bone sites in women receiving combined oral contraceptives

- and depot-medroxyprogesterone acetate for contraception. *Contraception*, vol.65, no. 6, pp.407-10.
- Weaver, C.M., Teegarden, D., Lyle, R.M., et al. 2001. Impact of exercise on bone health and contraindication of oral contraceptive use in young women. *Med Sci Sports Exerc*, vol.33, no. 6, pp.873-80.
- Westhoff, C. 2003. Depot-medroxyprogesterone acetate injection Depo-Provera[®]: a highly effective contraceptive option with proven long-term safety. *Contraception*, vol.63, no. 2, pp.75-87.
- Wetmore, C.M., Ichikawa, L., LaCroix A.Z., et al. 2008. Association between caffeine intake and bone mass among young women: potential effect modification by depot medroxyprogesterone acetate use. *Osteoporos Int*, vol.19, no.4, pp.519-27.
- World Health Organisation. 1983. Special Programme of Research, Development and Research Training in Human Reproduction. Task Force on Long-acting Systemic Agents for the Regulation of Fertility. Multinational comparative clinical evaluation of two long-acting injectable contraceptive steroids: Norethisterone enanthate given in two dosage regimens and depot medroxyprogesterone acetate: Final report. *Contraception*, vol.25, no.1, pp.1-11.
- World Health Organisation. 1994. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. *World Health Organ Tech Rep Ser*, vol.843, pp.1-290.
- World Health Organisation. 2002. Medical Eligibility Criteria for Contraceptive Use Third edition. Department of Reproductive Health and research, World Health Organization, CH-1211- Geneva 27, Switzerland.

World Health Organisation. 2004. Medical Eligibility Criteria for Contraceptive Use Third edition. Department of Reproductive Health and research, World Health Organization, CH-1211- Geneva 27, Switzerland.

World Health Organisation. 2006. Statement on hormonal contraception and bone health. Department of Reproductive Health and research, World Health Organization, CH-1211- Geneva 27, Switzerland. *Contraception*, vol.73, no.5, pp.443-444.